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Evaluation of antioxidant and nutritional properties of sago (*Metroxylon sagu* Rottb.) and its utilization for direct lactic acid production using immobilized *Enterococcus faecium* DMF78

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Abstract

Sago flour obtained using the *Argao* process (indigenous Philippine flour extraction process) was prepared into different mesh sizes (60, 100 and 200 mesh). The sieved flour was divided into fine (F) and course (C) fractions and were tested for percent process recovery, proximate analysis, total starch, total phenolic content, and antioxidant activity. Percent process recovery ranged from 77–89%. Crude fat content was less than 1% (w/w) for all samples while crude fiber content were significantly higher in the coarse fractions. Starch content(db) of the fine fractions were 88.31±1.78% (F60), 92.87±1.49% (F100), and 96.62±1.03% (F200) while coarse fractions had a total starch content(db) of 74.65±1.20% (C60), 68.66±0.96% (C100), and 64.67±1.14% (C200). Total polyphenol content of fine flour fractions (2.83–6.17mg GAE/g sample) was significantly lower than the course fractions (31.50–42.76mg GAE/g sample). Moreover, sago was used as substrate for lactic acid production. Agar, alginate and κ-carrageenan were tested as immobilization matrices for *E. faecium* DMF78. Alginate (3% w/v) extruded drop wise on 0.2M $CaCl_2$, and cured for 1hr was found to be the most efficient immobilizing matrix. The use of immobilized and free cells led to the production of 3.011g/L and 4.80g/L lactic acid after 24hrs, representing lactic acid productivity of 0.125 $g.L^{-1}h^{-1}$ and 0.200 $g.L^{-1}h^{-1}$ and lactic acid yield (conversion) of 25.42% and 39.76%, respectively.

Introduction

Sago flour is a cheap and abundant plant resource in Southeast Asia that is derived from the pith of *Metroxylon sagu*, growing extensively in the peat swamps of Malaysia, Indonesia, Papua New Guinea, and can also be found wild in marshy areas in the southern part of the Philippines. Starch accumulates in the trunk of the sago palm with a confirmed 25-ton per hectare per year of starch productivity (Ishizaki, 1997). So far, it has the highest record of productivity among starchy crops in the world. It has been consumed for thousands of years in Southeast Asia and the Pacific and has been an important source of dietary carbohydrate in Papua New Guinea.

The recent awareness of consumers toward healthy food has spurred the search of non-conventional flours in relation to dietary fiber content and potent antioxidants. The quest for sources like sago, banana, and squash flours had generated attention. In terms of color acceptability, the dominant off-white sago flour obtained by a natural process should not be judged as undesirable for it contains considerable amount of polyphenols (i.e. catechins) which are considered as source of antioxidant in food (Ding, 2006). However, there are no available literatures showing the yield and physicochemical properties of sago flour obtained using an indigenous process.

Aside from being a staple food, sago flour specifically its starch content has a wide variety of uses. To take full advantage of this product, there is a rising global interest in producing high value products from sago starch, such as high fructose syrup (Limbaga, 2007), dextrins (Wong *et al*., 2007), ethanol (Lee *et al*., 1987; Bandaru *et al*., 2006) and lactic acid (Toleco *et al*. 2016). Lactic acid is a valuable product that can be produced chemically (Demirci and Pometto, 1995) or by fermentation of carbohydrates (Vick Roy *et al*., 1983). In lactic acid fermentation, the most common substrate used is

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glucose, which is expensive. The production of lactic acid is therefore costly due to its process and high cost of glucose. In an effort to lower down the cost of production, studies are being conducted to find cheaper substrates and more cost-efficient methods of production. This study aims to characterize sago flour and utilize sago starch for the direct lactic acid production using immobilized *E. faecium* DMF78.

Materials and Methods

Chemicals and materials

Alginic acid and carrageenan (commercial grade, type 1, predominantly κ-carrageenan) were purchased from Sigma Co-Aldrich.Co., St. Loius MO, agar powder from Nacalai Tesque Inc., Japan and ρ-phenylphenol from Wako Pure Chemical Industries, Ltd. Analytical and HPLC grade reagents were used.

Sago flour processing

Sago was obtained from Argao, Cebu, Philippines. The sago log was debarked and the pith was made into thin strips and sun-dried for 3–4 days. The process is a local custom and shall henceforth be called the "*Argao* process". The dried strips were ground using a Waring™ Blender. The ground sago flour was first sieved through 20 mesh screen and was successively passed thru 60, 100 and 200 mesh screens to produce the desired flour samples. Two fractions were made in each mesh size: fine (F) and coarse (C) fractions. By definition, the fine fractions were those that passed through the sieve while the course fractions were retained above the sieve.

Proximate composition analysis

The different sago flour fractions were subjected to the following analyses: total moisture content (air-oven method, AOAC 925.10), crude ash/minerals (AOAC 923.03), crude fat (AOAC 920.39), and crude fiber (AOAC 984.04). The sum of all components will be deducted from 100 to determine the % nitrogen-free extract (NFE) of the sample.

Dietary fiber quantification

Dietary fiber was quantified by combining the values of carbohydrates and crude fiber of sago. Carbohydrates is computed as the total non-starch polysaccharides (i.e. polyphenols) and total starch. The following formulas were used for the computation:

$$\textit{Proximate Composition (100\%)} = MC + CF + Cfbr + CA + CP + Cbd$$

$$\textit{Carbohydrates (Cbd)} = \textit{Total Starch} + \textit{Non-starch polysaccharides}$$

$$\textit{Dietary Fibre} = Cbd + Cfbr$$

where MC, CF, Cfbr, CA, CP and Cbd refer to moisture content, crude fat, crude fiber, crude ash, crude protein, and carbohydrates (by difference) respectively.

Total starch content

Total starch determination was done using anthrone method as described by McCready *et al*. (1950).

Total phenolic content

Total phenolic content was measured according to Singleton and Rossi (1965) with modifications. Extraction of phenolic compounds was done using 1g and 3g course and fine sago flour samples in 50mL 80% aqueous methanol placed in an ultrasonic bath (Elma Ultrasonic LC30 H; Melrose, Park, USA) for 20 mins. Two mL aliquot of the extracts was centrifuged for 5mins at 20,000 x g using CT 15RT refrigerated centrifuge (Techcomp, Ltd., Shanghai, China). One mL of the centrifuged aliquot was added into 100mL volumetric flask, containing 9mL deionized water. One mL of Folin-Ciocalteau's phenol reagent was added to the mixture and vortexed. After 5mins, the sample was added with 10mL 7% Na_2CO_3 solution, diluted to volume using deionized water, and incubated for 90 mins at room temperature. The absorbance against prepared reagent blank was determined at 750nm using UV-Vis Spectrophotometer Shimadzu (1601) (Kyoto, Japan) with gallic acid as standard. Total phenolic content of sago flour was expressed as mg gallic acid equivalents (GAE)/g sample.

DPPH radical scavenging activity

Free radical scavenging activity was determined according to Budzianowski and Budzianowska (2006) with some modifications.

Absolute methanol (100mL) was added to the flour sample (10g for fine fractions and 2.5g for course fractions as samples). The mixture was shaken at 250rpm in a rotary shaker for 30mins, placed in an ultrasonic bath for 15mins, filtered, and subjected to vacuum rotary-evaporator at 55°C. The dried extract was reconstituted with 25mL absolute methanol that served as the stock solution.

Sago flour extract (200µL) sample solutions in methanol was mixed with 2.8mL of 100µM DPPH [1,1-diphenyl-2-picryl-hydrazyl (α, α-diphenyl-β-picrylhydrazyl)] in absolute methanol solution. The

absorbance was read after 30 mins at 517nm. The free radical DPPH scavenging (i.e. reduction) activity was calculated using the equation:

$$Activity\ (\%\ DPPH\ Reduction) = \left(\frac{A - Ax}{A}\right) x\ 100\%$$

where A is the absorbance of DPPH solution with absolute methanol (control), Ax is the absorbance of DPPH solution with tested sago flour fraction or Vitamin E (positive control) solution. The antiradical activity, IC_{50}, defined as the concentration of a sample showing 50% DPPH radical scavenging activity, was determined using Vitamin E as the positive control.

Ferric reducing antioxidant power (FRAP)

The antioxidant activity of a 20:1 (mg/mL) 80% methanol extraction for fine fractions and 2:1 (w/v) (mg/mL) for course fractions was determined by FRAP method according to Réka Szőllősi and Varga (2002). Forty µL of sample was added with 1.2mL FRAP working solution, vortexed, and read at 593nm after 10mins.

Whole-cell immobilization preparation of inoculum

E. faecium DMF78 was obtained from the Department of Food Science and Chemistry, University of the Philippines Mindanao. A loopful of the 24-hr old culture was inoculated onto modified MRS broth (100 mesh sago starch as substrate instead of glucose) and was incubated at 30°C for 24hrs.

Preparation of cells for immobilization

Three hundred mL of modified MRS broth was inoculated with 10% fermentation starter, incubated at 30°C for 16hrs, and cells were harvested by centrifugation at 4,500 x g for 20min at 4°C. The supernatant was discarded and the cells were suspended in sterilized distilled water to obtain a 40% cell suspension, wet weight basis.

Immobilization

Whole-cell immobilization in agar and alginate was done according to Adinarayana *et al*. (2005) with modifications. For agar as the immobilizing matrix, 5mL of cell suspension was added to 2% agar with 0.9% sodium chloride (NaCl) maintained at 40°C, with gentle orbital shaking. The resulting suspension was then poured into sterile petri dish and was allowed to harden for 30mins. The resulting gel was cut into blocks (5mm x 5mm x 5mm) and soaked in sterile 0.1M phosphate buffer (pH 7.0) for one hour at 4°C, washed 3x to remove extraneous microbial cells with sterile distilled water and stored at 4°C until use.

In using κ-carrageenan as the immobilizing matrix, 5mL cell suspension was mixed with 50mL sterile 2.5% κ-carrageenan solution. The resulting suspension was poured onto a sterile petri dish and was allowed to solidify for 30mins. The resulting gel was cut into cubes (5mm x 5mm x 5mm) using a sterile scalpel, allowed to harden by soaking in 0.3M potassium chloride (KCl) solution for 1hr at 4°C, washed with sterile distilled water 3x, and stored in sterile distilled water at 4°C until use.

In using alginate as the immobilizing matrix, 3% sterile sodium alginate solution was added with cell suspension in a 1:1 ratio and mixed. The resulting suspension was extruded drop wise from a 20cm height into a 0.2M calcium chloride ($CaCl_2$) solution using a peristaltic pump with a 1mm tubing at a flow rate of 170mL/hr. The beads were kept in solution at 4°C for curing (1hr), washed with sterile distilled water 3x, and stored in sterile distilled water at 4°C until use.

Optimization of different parameters per immobilization matrix

The effect of agar concentration (1%, 1.5%, 2% and 2.5%), agar quality (Nacalai Tesque™ and Hi-media™ agars), and sodium chloride (NaCl) concentration (0%, 0.7% and 0.9%) were tested to determine the optimum parameters for the use of agar as an immobilization matrix. The gels were prepared as described earlier with phosphate buffer pH 7.0.

The effect of extended curing time (1 hour) with calcium chloride ($CaCl_2$) as curing agent and the effect of potassium chloride (KCl) concentration (0.1M, 0.3M, 0.5M and 1M) were tested to determine the optimum parameters for the use of κ-Carrageenan as an immobilizing matrix. The effect of alginate concentration (2%, 3%, 4%, 5% and 6%), calcium chloride ($CaCl_2$) concentration (0.1M, 0.2M, 0.3M and 0.4M), and curing time (1, 2, 4, 6 hrs) were tested to determine the optimum parameters for the use of alginate as an immobilizing matrix. The beads were prepared as described earlier using 0.2 M calcium chloride ($CaCl_2$) as the catching solution.

Estimation of residual starch

Residual soluble starch was analyzed using iodine-starch complex colorimetric method according to Nakamura (1979). The amount of starch was estimated using soluble starch as standard.

Estimation of reducing sugar

Estimation of residual sugar was done according to Borel *et al*. (1952). The amount of sugar was estimated using glucose as standard.

Lactic acid determination

Lactic acid determination was done using high performance liquid chromatography (HPLC, Prominence Ultrafast Liquid Chromatography (UFLC), Shimadzu, Kyoto, Japan) equipped with Supelco Gel C-610H column operated at 50°C, with a mobile phase of 5mM sulfuric acid (H_2SO_4) and a flow rate of 1mL/min. Samples were pre-filtered using 13mm, 0.45mμ Millipore filter.

Direct lactic acid production from sago starch

Batch fermentation was carried out in a 125mL Erlenmeyer flask. A 27-mL modified MRS medium sterilized in an autoclave at 15psi (~121°C) for 15mins was added with 10% of the immobilized cells. Fermentation was carried out at 30°C for 24hrs with agitation (100rpm).

Lactic acid productivity and yield

Lactic acid productivity and yield from sago starch was calculated according to Shen and Xia (2006) using the following equations:

$$\text{Lactic acid productivity}\left(\frac{g}{L \cdot hr}\right) = \frac{\text{concentration of lactic acid produced}\left(\frac{g}{L}\right)}{\text{fermentation time (hr)}}$$

$$\text{Lactic acid yield} = \left\{\frac{\text{concentration of lactic acid produced}\left(\frac{g}{L}\right)}{\text{starch utilized}\left(\frac{g}{L}\right)}\right\} \times 100\%$$

Estimation of cell wash out

Each optimized immobilized matrix was tested for the estimation of cell wash out. A sample of 0.5mL fermentation media was aseptically withdrawn for pour plate count of viable cells using modified MRS agar. Samples were obtained at an 8-hr interval for 24hrs. Plates were incubated at 30°C and colonies observed with zones of clearing were counted as colony forming unit per mL (CFU/mL).

Results and Discussion

Percent recovery and sago flour characterization

The yield recovery (%) of sago flour obtained using the *Argao* process was evaluated. Successive sieving (from 60 to 200 mesh) of ground sago flour resulted to a yield recovery (%) of 87.23±2.15% (F60), 80.12±3.38% (F100) and 65.16±3.66% (F200) for fine sago flour while coarse sago flour recovery accounts to 5–14.40% (C60-C200) of the original sago flour weight. The traditional wet processing techniques for sago flour extraction had been constantly revised to raise yield recovery (%). To achieve this, additional processing steps are necessary, which leads to increase production costs. The *Argao* process is an alternative way to process sago flour. If the sago piths are readily processed and dried (with correct drying techniques) the additional costs on wet processing of sago flour (i.e. water, pH regulation and bleaching agents) will be circumvented. The resulting sago flour has off-white to light pink color that is desirable to consumers (Ozawa *et al*., 1991; Karim *et al*., 2008).

Physicochemical properties of sago flour

The mean % composition of sago flour passed through different mesh sizes is shown in Table 1. The fine flour fractions were found to contain 14.00-15.00% moisture, 1.60-2.40% ash(db), 0.18–0.22% fat(db), 0.07–1.73% fiber(db); 86.50–97.70% total starch(db), and 0.30–0.80% total polyphenols(db). The light brown color of the flour produced may be attributed to the polyphenols present. The F60 and F100 fractions were found to have the same fiber content while the phenolic content of F100 when compared to F60 and F200 was not significantly different.

The sago flour obtained from the "*Argao* process" had lower starch content and higher non-starch components in comparison with mungbean, adzuki bean, corn, wheat, and potato starches and flour. Non-starch components of the aforementioned starches and flours are <1.0% (Abdel-Rahman *et al*., 2008). Furthermore, the F200 sago flour obtained using the *Argao* process was almost similar in proximate composition to the starch produced by Karim *et al*. (2008) using wet starch extraction. The only differences were: (1) ash content of F200 was higher in this study and (2) flour color was light brown as compared with the white sago starch reported by Karim *et al*. (2008).

Flour ash is related to milling extraction and is used as a measure of flour quality and an indication of milling efficiency (Kawamura *et al*., 2008). The presence of ash is a widely accepted index of refinement in foods. This can be used to crudely know the purity of the flour or starch (Pomeranz and Meloan, 1994). In this study, crude ash was negatively correlated with starch content (r = -0.9688, t > 0.005). Specific crude ash values indicate the starch content of the sago flour. The higher the crude ash, the lesser is the starch content of the flour. The sago flour coarse fractions had high crude fiber contents. Fiber content can be used as a feed value index for livestock feeds. It can also be used as a direct index of flour purity as compared to color or ash (Pomeranz and Meloan, 1994).

Table 1. Mean percent composition (100%) and antioxidant activity of different sago flour mesh sizes.

Mesh Size[x]	Physicochemical Observations								Antioxidant Activity	
	MC	CA(db)	CF(db)	CFbr (db)	CP(db)[y]	TS(db)	TP(db)	TDF(db)[y]	DPPH Radical Scavenging Activity IC_{50} (mol. Vit. E equivalents/g extract)[z]	Ferric Reducing Antioxidant Power mmol Fe (II)/g sample
C60	14.23 ± 0.04^{bc}	4.76 ± 0.07^{a}	0.22 ± 0.01^{a}	7.29 ± 0.17^{a}	0.22 ± 0.04	74.64 ± 1.2^{a}	3.68 ± 0.15^{a}	9.40 ± 1.08	2.63 ± 0.19^{a}	11.08 ± 0.68^{a}
C100	14.24 ± 0.06^{ab}	6.24 ± 0.02^{b}	0.19 ± 0.01^{a}	9.04 ± 0.35^{b}	0.22 ± 0.04	68.66 ± 0.96^{b}	4.55 ± 0.26^{b}	11.32 ± 1.49	2.22 ± 0.43^{a}	12.40 ± 0.37^{a}
C200	14.21 ± 0.02^{ac}	8.96 ± 0.05^{c}	0.20 ± 0.00^{a}	9.56 ± 0.20^{c}	0.22 ± 0.04	64.67 ± 1.14^{c}	4.99 ± 0.06^{c}	11.62 ± 1.21	2.46 ± 0.41^{a}	17.49 ± 1.09^{e}
F60	14.38 ± 0.04^{df}	2.34 ± 0.02^{d}	0.20 ± 0.01^{a}	1.66 ± 0.07^{d}	0.22 ± 0.04	88.31 ± 1.78^{d}	0.72 ± 0.06^{d}	6.77 ± 1.71	2.54 ± 0.32^{a}	1.77 ± 0.00^{bc}
F100	14.38 ± 0.03^{ef}	1.97 ± 0.01^{e}	0.20 ± 0.02^{a}	1.20 ± 0.04^{d}	0.22 ± 0.04	92.87 ± 1.49^{e}	0.52 ± 0.04^{de}	3.24 ± 1.48	2.96 ± 0.48^{a}	1.44 ± 0.11^{cd}
F200	14.43 ± 1.26^{de}	1.58 ± 0.01^{f}	0.20 ± 0.02^{a}	0.08 ± 0.01^{e}	0.22 ±0.04	96.62 ± 1.03^{f}	0.33 ± 0.02^{e}	1.19 ± 1.01	3.06 ± 0.27^{a}	0.84 ± 0.01^{bd}
Soluble Starch	12.23 ± 0.02^{g}	0.13 ± 0.00^{g}	-	-	-	97.78 ± 1.22^{f}	-	-		

Moisture content (MC); crude ash (CA); crude fat (CF); crude fiber (CFbr); crude protein (CP); total starch (TS); total polyphenol (TP); total dietary fiber (TDF)

[x]Mesh size = Coarse fractions (C) and Fine fractions (F) of sorghum flour samples.

[y]Assumed value for protein from Karim *et al*. (2006) and TDF by difference of all assessed values (db)

[z]C_{50}: the inhibitory concentration of antioxidant decreasing initial DPPH radical by 50%. IC_{50} values are given as mean±SD (n=2).

Means within a column with the same superscript are not significantly different (HSD Test) (q=0.05).

Ferric reducing antioxidant power

The half-inhibition concentration (IC_{50}) required to decrease an initial DPPH concentration by 50% is presented in Table 1. Results showed that the coarse and fine fractions have more or less the same batch of antioxidants. The FRAP values obtained in coarse and fine sago flour fractions were greater than those reported in berries (i.e. raspberries and strawberries, 2.00 – 5.50 mmol Fe (II)/100 g, wine (red, rose and white), 0.40-3.00 mmol Fe (II)/100 ml, and cereals, 0.59 mmol Fe (II)/L (Pellegrini *et al*. 2003). It is interesting to note that the coarse fractions of sago flour were good sources of antioxidants. Sago had always been branded as the poor man's crop and is being marketed cheaply. This study showed that antioxidative benefits available in elite foods such as berries and grapes are also present in sago flour.

Polyphenol content of sago compared to other foodstuff

A number of foodstuffs have been studied for its putative radical-scavenging activity that most people believed to have exerted several health-promoting functions (Teow *et al*., 2007). Regardless of the varietal differences of bioactive components in most foods, the total polyphenol content crudely measures the strength of a food in combating oxidative stress. Table 2 shows the total phenolic content of a few known foodstuffs, containing high amounts of phenolics, in comparison with sago flour. The course sago flour fractions have high phenolic content in comparison to known food products that have antioxidative capability.

Table 2. Total polyphenol content of foodstuff in comparison with sago flour (wet wt.).

Samples	Total Polyphenol Content	Author(s)
	mg GAE/g sample	
Sago (C60)	31.56±1.25	This study
Sago (C100)	39.00±2.25	This study
Sago (C200)	42.76±0.47	This study
Sago (F60)	6.17±0.49	This study
Sago (F100)	4.58±0.54	This study
Sago (F200)	2.83±0.14	This study
Quinoa seeds	3.75	Pasko *et al*., 2009
French grapes	1.96	Brat *et al*., 2006
French artichoke	3.21	Brat *et al*., 2006
Walnut cultivars	16.04	Anderson *et al*., 2001
U.S.A. wheat bran[z]	1.35-1.98	Guttieri, 2002
French strawberries	2.64	Brat *et al*., 2006

Table 2 (Cont.)

Brazilian apple peels	6.41	Vieira *et al*., 2009
Czech Republic malt	1.60-2.70	Dvořáková *et al*., 2008
Czech Republic barley	0.60-1.10	Dvořáková *et al*., 2008
Durian cultivars (i.e. Chani)	271.50-361.40	Toledo *et al*., 2008

[z]expressed as mg GAE/ g dry weight.

Proposed sago flour grading

The primary variables for grading "*Argao*" made sago flour were total starch, crude ash and crude fiber content. Results shown in Table 1 suggests that the three variables are independent from each other and have significantly different values when compared to the mesh size of course and fine fractions of sago flour.

Starch and crude ash contents have been found to be inversely proportional ($r = -0.9688$, $t > 0.005$). The ash values can be used to approximate the grade of the resulting sago flour. On the other hand, the fat and protein content values of sago flour were usually lower than 0.2% (Karim *et al*., 2008). Thus, they were not included as criteria for sago flour obtained using the "*Argao* Process". It was also proposed, that the moisture content of the sago flour fractions should be regulated at 10-12%. The said values were in coordination with current cassava and sago flour standards (Karim *et al*., 2008; Singhal *et al*., 2007). The moisture content range was proposed to prevent mold growth, bacterial contamination and insect infestation.

Production by immobilized E. faecium DMF78

E. faecium DMF78 is a novel amylolytic lactic acid bacterium that has been reported to have the ability to directly convert starch into L-(+)-lactic acid that prefers sago starch as substrate. To further improve the lactic acid production, the immobilization of this bacterium was investigated.

Optimization of immobilizing matrices

Three matrices were used to compare its efficiency if used as an immobilizing matrix for *E. faecium* DMF78 for the direct lactic acid production from sago starch. Agar, alginate, and κ-carrageenan were initially optimized (data not shown). The use of these matrices were evaluated based on the amount of lactic acid produced, residual starch, and reducing sugars. Results of experiments using optimized immobilized matrices showed that the use of alginate leads to a lactic acid production of 3.011 g/L (Figure 1a). It also showed that it has highest reducing sugar and lowest residual starch reading.

Table 3 shows the comparison of results for the kinetic parameters of the batch fermentation of *E. faecium* DMF78 using three immobilization matrices. Results show that of the 20 g starch feed, there is a 2 to 5 g/L residual reducing sugar. This could mean that immobilized *E. faecium* DMF78 did not actively convert sago starch to lactic acid. Starch utilization was only 23-25% while the control has a 39% conversion.

Low levels of lactic acid production may be due to the failure of the microorganism to convert all the starch utilized directly into lactic acid and that the starch remains as free glucose in the medium. There can be a limited diffusion of substrate (starch) into the inner region of the gel thus limiting the interaction between the substrate and the enzyme converting it to lactic acid. According to Gikas and Livingston (1997), cells located close to the nutrient supply are likely to maintain higher quality and activity

Table 3. Kinetic parameters of the batch fermentation of *E. faecium* DMF78 using three immobilized matrices and incubated for 24hrs at 30°C with agitation.

Parameters	Immobilizing Matrices			
	Agar	Alginate	k-Carrageenan	Control: Free cell
Starch concentration at 0hr (g/L)	20	20	20	20
Cell wash-out/microbial growth after 24hrs (CFU/mL)	1.56×10^{7b}	8.47×10^{6c}	2.35×10^{7b}	8×10^{7a}
Starch concentration after 24hrs (g/L)	8.84	8.16	8.25	7.93
Residual Reducing Sugar (g/L)	2.72	4.10	3.59	-
Starch utilized (g/L)	11.16	11.83	11.75	12.07
Starch utilization (%)	55.8	59.15	58.75	60.37
Lactic acid concentration in broth after 24hrs (g/L)	2.85^{bc}	3.01^{b}	2.78^{c}	4.80^{a}
Volumetric lactic acid productivity ($g.L^{-}1.h^{-1}$)	0.119	0.125	0.116	0.200
Lactic acid yield (%)	25.53	25.44	23.66	39.76
Starch conversion efficiency ($g.g^{-1}.h^{-1}$)	0.018	0.017	0.017	0.017

* Means within a row with the same superscript are not significantly different (HSD Test) (q=0.05).

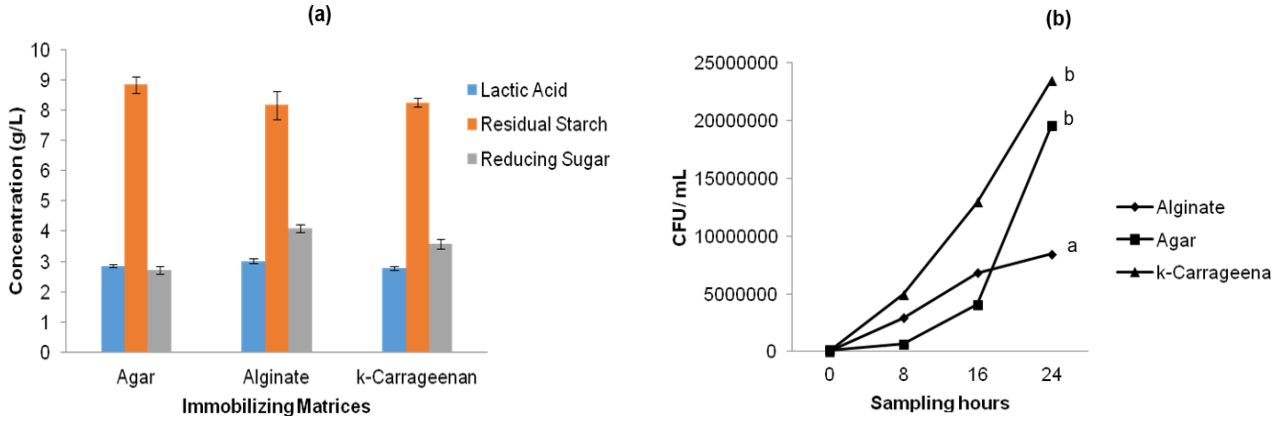

Figure 1. (a) Comparison of lactic acid, reducing sugar and residual starch for the 3 immobilizing matrices; (b) Estimation of cell wash out for 24 hours. Different letters within a column mean statistical difference at 95% confidence level.

in comparison with cells located relatively further inside, leading to differences in quality/activity of the immobilized cell population. Oxygen transfer to immobilized cells may also differ.

Estimation of cell wash-out

Cell wash-out was studied in order to determine the occurrence and extent of leakage in the particular immobilizing matrix. In this part of the study, the fermenting medium was examined for viable plate count. Moreover, cell wash out may also be attributed to the cells that was only adsorbed in the immobilizing matrix. Figure 1b shows that as the fermentation proceeded, the amount of cell wash-out also increased. At the end of 24 hours, among the three matrices, alginate showed the least amount of cell leakage. Cell leakage can also be attributed for its contribution to the lactic acid produced by each matrix. More so, that the CFU/mL increases as fermentation time increases.

Conclusion

The study aimed to produce *Argao* processed sago flour of various mesh sizes, for characterization and utilization for the direct lactic acid production using whole-cell immobilization technique. For the sago flour characterization, the flour produced was from 60, 100 and 200 mesh, respectively. Two fractions were made in each mesh size: fine (F) and coarse (C) fractions. The six fractions were evaluated based on yield percent recovery, proximate analysis, total starch content, total polyphenols, and antioxidant activity/capacity. Results have shown that yield % recovery of the process ranges from 77–89%. Crude fat content was less than 1% (w/w). Crude fiber content were significantly higher in coarse fractions (7.12–9.76%(db)) than in fine fractions (0.07–1.73%(db)). The total starch content was also lower in coarse fractions (63.50–75.90%(db)) than in fine fractions (86.50–97.70%(db)). The total polyphenol content and antioxidant activity of the *Argao* processed sago flour was comparable in value to known antioxidative and healthy food.

The light brown sago flour produced by the *Argao* process was different from the commercially available sago flour and starch (the standard white flour/starch). The flour retained the polyphenol content and antioxidative property in exchange to a generally acceptable appearance. For the utilization of sago flour as substrate for the direct lactic production using immobilized *E. faecium* DMF78, tests for residual reducing sugars, residual starch and estimation of cell wash out on three matrices bore out the better performance of alginate. HPLC analysis of lactic acid further confirmed that alginate was the best immobilizing matrix for *E. faecium* DMF78. The average lactic acid produced using alginate as the immobilizing matrix was 3.011 g/L after 24 hours of fermentation.

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