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PRODUCTION OF SINGLE-CELL PROTEIN FROM DARJEELING MANDARIN PEELS USING *ASPERGILLUS NIGER* AND *ASPERGILLUS ORYZAE*

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ABSTRACT: Single Cell Protein (SCP) refers to the dead, dried cells of various microbes, including bacteria, yeast, algae, and fungi. This type of biomass can be obtained from economically cheap substrates like agro-wastes as a non-conventional proteintious source for human or animal feed. The bioconversion of Darjeeling mandarin peels into single-cell protein (SCP) was performed using *Aspergillus niger* and *Aspergillus oryzae*, by submerged fermentation. The study revealed that supplementing Darjeeling mandarin peels with inorganic nitrogen and glucose as carbon sources improved the fungal biomass and single-cell protein production. *Aspergillus niger* showed the highest biomass and protein yield of 2.53 ± 0.005 gm/l and 1.25 gm/l or $43.57 \pm 0.00\%$, respectively. The present study revealed that fungal biomasses had shown crude fat (ranging from to 1.16 gm/l), crude fiber (ranging from 0.06 ± 0.001 to 1.97 gm/l), and total genomic DNA content (from 1.85 ± 0.001 to $2.20 \pm 0.001\%$). Maximum ash content was observed by *A. niger* biomass in GMPM (3.89%). *A. niger* converted sugar 71.4% to yield biomass. *A. niger* and *A. oryzae* were useful for producing fungal biomass from Darjeeling mandarin peels.

INTRODUCTION: Protein is one of the major nutrients which helps in different cellular activities by catalyzing various chemical reactions occurring in the cell, providing structural elements of cells to form tissues, creating antibodies to living bodies from diseases, and controlling the activity of genes to regulate gene expression. In the early fifties, an alternative source of proteins was introduced as single-cell protein due to the lack of availability of enough protein sources ¹.

Single-cell protein (SCP), also known as microbial protein, was developed as an unconventional protein source of food and animal feed ². The term SCP was introduced in 1966 by Carol L. Wilson. It refers to dried dead cells of edible unicellular microorganisms like algae, yeasts, fungi, or bacteria which may be utilized as an ingredient or a substitute for protein-rich foods, flavour enhancers, and fat-binding agents ³.

Fruit processing wastes can cause different environmental hazards, but they are mostly biodegradable and rich sources of various nutrients for microbial growth. Utilizing these wastes as a potential substrates for producing SCP will help decrease the environmental pollution. Various studies have shown that agricultural wastes like watermelon peels, and pineapple peels could be

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used as beneficial substrates for SCP production⁴. Orange peel wastes are rich sources of cellulose (9.21%), hemicelluloses content (10.5%), and pectin content (42.5%). Hence, these waste materials are useful as raw materials for producing SCP in animal feed, which exhibits low pectin and crude fiber contents^{5,6}. Darjeeling mandarin peels (*Citrus reticulata*) also comprise high carbohydrate content and low protein and fat contents⁷. Bioconversions of these mandarin peels using different fungi to develop SCP can be a cost-effective process due to its climate-independent nature, the requirement of less production area, reduced generation time, and usage of agricultural wastes for the production of SCP⁸. Utilization of SCP using fungal strain has been proven more efficient than bacterial and algal SCP due to their low operating, isolating and recovery costs⁹. Many studies were carried out on the utilization of sweet orange peels (*Citrus sinensis*) for SCP, only few studies were performed using mandarin peels¹⁰.

In this work, peels of Darjeeling mandarin were used as raw material for submerged fermentation to produce fungal biomass using two fungi *Aspergillus niger*, *Aspergillus oryzae*. This research work aimed to determine the bioconversion efficiency of these fungal strains to produce SCP using the carbohydrate content of mandarin peel and the effect of nutrient supplementation on the production of SCP.

MATERIALS AND METHODS:

Microorganisms: Two filamentous fungal strains *Aspergillus oryzae* MTCC 3782 and *Aspergillus niger* MTCC 9687, were obtained from the Microbial Type Culture Collection (MTCC, Chandigarh, India) and chosen for the bioconversion procedure. Czapek yeast and malt extract agar were prepared for *Aspergillus oryzae* and *Aspergillus niger*, respectively, and autoclaved at 121°C for 15 min. The sterilized media were poured into sterilized test tubes for slant preparation, and the slants were inoculated and incubated at 30°C for 48 h to obtain sufficient growth. After the incubation period, the fungal strains were stored at 4°C. Each strain was subcultured in every 2 weeks and incubated at 30°C. Suspension of spores of each of the two fungal strains was prepared in 15% (v/v) sterile glycerol and kept at -30°C for further use¹¹.

Reagents Required: All components were procured from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Other chemicals were obtained from Merck, India, and analytical grade.

Collection of Fruit Peel Wastes: The fresh impurities of mandarin fruits were collected from Darjeeling, West Bengal, India. The fruit peels were washed gently with tap water to remove the dust and contaminants. The peels were weighed and completely oven dried at 65°C and ground to powder with mortar and pestle. The ground samples were stored in sterile transparent polyethylene bags and at 4°C for further use.

Proximate Compositions of Darjeeling Mandarin Peels: The moisture content was determined based on the standard Association of Official Analytical Chemists (AOAC) method¹². The Protein content was calculated using Folin Lowry method¹³. The total sugar was estimated by Anthrone method¹⁴. Total fat was extracted with a mixture of chloroform-methanol (1:1, v/v) according to AOAC (1951) method¹⁵. The ash content of the sample was determined by igniting it in a muffle furnace at 550°C¹⁶. All analytical determinations were performed in triplicate.

Preparation of the Inoculums: 2 days old cultures of two fungi were washed with 25 ml of sterile distilled water to prepare the inoculums. The spores of the fungal suspensions were rubbed, and the final concentration of the inoculums was maintained at 10⁶ spores per ml¹⁷. The inoculums were stored at 4°C for further use.

Substrate Preparation: 4 gm of dried peel powder was poured into a sterilized 250 ml Erlenmeyer flask. 100 ml of distilled water was mixed with the peel powder and boiled in a water bath for 1 hr at 75°C for juice extraction. It was then filtered through cheesecloth. The filtrate was autoclaved at 121°C for 15 minutes and cooled to room temperature. The resulting sterile filtrate of fruit peel was used as the substrate for biomass production.

Submerged State Fermentation: Submerged state fermentation was carried out into 250 ml Erlenmeyer flasks using each of the two fungal strains. The fungal strain was used according to a constant substrate concentration, inoculum size and

fermentation condition. Three media types were prepared for fermentation using mandarin peel extract as substrate. The first experimental medium was designated as control mandarin peel media (CMPM) which consisted of only peel extract as the source of carbon and nitrogen. The second medium was labelled as supplemented mandarin peel medium (SMPM) comprised of $(\text{NH}_4)_2\text{SO}_4$ (2 gm), KH_2PO_4 (1gm), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 gm), NaCl (0.1 gm), CaCl_2 (0.1 gm) and supplemented with 20 ml fruit peel extract. The next medium was named glucose-supplemented mandarin peel medium (GMPM) with similar compositions of SMPM and additional glucose (2 gm/l) as sugar supplementation. 98 ml of each media was poured into 250 ml Erlenmeyer flask, and the final pH was maintained at 6.5 using 1N NaOH. All the flasks were autoclaved at 121°C and 15 psi pressure for 20 minutes, and the flasks were then cooled to room temperature. 2 ml inoculums from suspensions of each of the two filamentous fungi suspensions were transferred aseptically into each flask. The flasks were then incubated at 28°C under constant conditions for 11 days for sufficient fungal growth. The biomass and other parameters were determined during the fermentation period of 6-11 days. All experiments were performed in triplicate to reduce experimental error.

Harvesting and Compositional Analysis of Single Cell Protein: After fermentation, fungal mat from culture broth was vacuum filtered on filter paper. The collected mat on the filter paper was washed with sterile distilled water and was oven dried at 55°C for constant weight. The total amount of biomass collected was estimated by calculating the difference between the weight before and after drying using the following equation¹⁸.

$$\text{Biomass} = W_2 - W_1$$

W_2 : weight of filter paper and mat before drying;

W_1 : weight of filter paper and biomass after drying.

Compositional Analysis of the Harvested SCPs:

The proximate compositions of resulting biomass, such as moisture content, total carbohydrate content, protein content, lipid content, ash content, and fiber content, were evaluated by the same procedure as described earlier.

Estimation of Total Genomic DNA: Total genomic DNA was determined according to the study of Hassett *et al.* (1992) with little modification¹⁹. Biomass produced from each medium was treated twice with a solution of 50 mM Tris-HCl (pH 8.0), 5 mM EDTA (pH 8.0), and 50 mM NaCl. The solution was kept at 55°C for 30 min, and 10% SDS and 10 µl of 10 mg/mL proteinase K enzyme were added to the solution. It was again 55°C for 10 min. This solution was washed with a mixed phenol: chloroform: isoamyl alcohol in the 25:24:1 ratio. The extraction procedure was performed again, and the supernatant was collected. The supernatant was mixed with one volume of 4 M ammonium acetate solution and two volumes of isopropanol. The solutions were centrifuged at 14000 rpm, and the DNA precipitate was separated with cold ethanol. It was again centrifuged, and the pellet was again collected and air-dried.

Determination of Microbial Efficiency of Substrate Sugar Conversion: To determine microbial efficiency of each of the two fungal strains used to convert fermentable sugar to fungal biomass after the 9-day bioconversion period was assayed according to the method described by Saheed *et al.*, 2016¹⁸. The equation was as follows-

$$\text{Conversion efficiency} = (I_o - I_f / I_o) \times 100$$

I_o : initial amount of metabolizable sugar;

I_f : final amount of metabolizable sugar.

Determination of Substrate Utilization Constant:

The production of SCP is inversely related to the metabolism of substrate sugar in the fermentation. The substrate sugar utilization constant (K_u) was calculated according to the following equation-

$$K_u = (B_2 - B_1) \times (S_1 - S_2)$$

K_u : Substrate sugar utilization constant; B_1 : initial fungal biomass; B_2 : final fungal biomass; S_1 : initial substrate sugar content; S_2 : final substrate sugar content

RESULTS AND DISCUSSIONS:

Proximate Composition of Darjeeling Mandarin Peels: The chemical composition of mandarin peels was described in Table 1. It revealed that

Darjeeling mandarin peels contained 8.36% moisture. Ekta Singh Chauhan et al (2018) study reported that steamed sundry kinnow peel powder contained 13.30% moisture²⁰. The present study illustrated that peel wastes contained good amounts of carbohydrates (72.2%), slightly lower than the Japanese mandarin peels evaluated by N. Nishio *et al.*, (1981). It was found that steamed sundry kinnow peel powder contained 73.80 % carbohydrate content. Feumba Dibanda Romelle *et al.*, 2016 also found that dry peel of sweet orange (*Citrus sinensis*) retained 53.27% carbohydrate²¹.

Table 1 showed that the protein content of Darjeeling mandarin peels was found to be 4.50±0.04%, much lower than sweet oranges (9.73%)²¹. Japanese mandarin peel also contained

6.20% protein. In the present study, ash content was higher (4.84±0.01%) than mandarins of Japanese origin (2.20%). The Ash content of the peel wastes represents the presence of organic matter, which was needed for the growth of fungi in producing SCP.

Table 1 revealed that the fat content of Darjeeling mandarin peel was 3.40%. The fat content in kinnow mandarin peels was found to be 1.235 %²¹.

The current work showed that Darjeeling mandarin peel wastes had enough carbohydrate, protein, lipid and moisture content for fungal growth. They can be used as a suitable substrate for microbial fermentation to produce single-cell protein.

TABLE 1: PROXIMATE ANALYSIS OF THE DARJEELING MANDARIN PEELS (IN 100G)

Sample	Moisture (%)	Carbohydrate (%)	Protein (%)	Fat (%)	Ash (%)
Raw mandarin peel	8.36±0.03	72.2±0.52	4.50±0.04	3.40±0.02	4.84±0.2

All values are the mean ± Standard Deviation of triplicates

Effect of Nutrient Supplementation on Fungal Biomass and Protein Production: **Table 2** revealed that biomass yields of both the fungi were directly proportional to the progression of the fermentation process.

It was found that the highest biomass production was observed on 11th day of fermentation due to the maximum spore formation of both fungi. In the control mandarin peel medium (CMPM), *A. niger* showed maximum fungal biomass yield (gm/l) followed by *A. niger* (0.92±0.002gm/l) at the 11th day of fermentation. In contrast, the minimum amount of biomass was recorded for *A. oryzae* (0.19±0.002gm/l) at the 6th day.

Fungal biomass produced in each SMPM media was increased due to the addition of various nutrients. The highest amount of biomass production (1.22±0.001gm/l) was observed in the SMPM medium of *A. niger* on the 11th day. In glucose-supplemented mandarin peel medium, the highest biomass yield was also recorded in the case of *A. niger* (2.53±0.005gm/l) on the 11th day of fermentation. The study of M. M. Abarshi *et al.*, (2017) revealed that the nutrient supplementation and glucose addition in the fermentation media increased the biomass production of *Saccharomyces cerevisiae* from watermelon and

pineapple peels. Similar results were also observed in the study of A. K. Mondal *et al.*, (2012). The present investigation also reported that the SCP production by the two fungi was increased gradually up to 9 days of fermentation and thereafter decreased.

The study also clearly showed that the highest amount of fungal protein was produced by *A. niger* (2.85 gm/l or 43.57±0.00%) on the 9th day of fermentation grown in glucose-supplemented mandarin peel medium. Similar findings in SCP production were reported in the study of Yakoub and Umar (2010) from agricultural wastes using *Penicillium expansum*²².

The protein content of *A. niger* biomass produced in supplemented mandarin peel medium (SMPM) medium at 9th day of fermentation was 1.03 gm/l or 36.01±0.02%, which was higher than control mandarin peel medium (CMPM) (0.92 gm/l or 32.13±0.01%). Due to the low content of nutrients in the control medium, the amount of fungal biomass protein was reduced. The study of A. K Mondal *et al.*, (2012) also reported that the supplemented fruit hydrolysates medium yielded low yeast biomass of *Saccharomyces cerevisiae* and low protein (17.47%) content than glucose-supplemented fruit hydrolysate medium. The study

of Abarshi *et al.* (2017) also involved the effects of nutrient supplementation on SCP production from fruit peels of watermelon and pineapple using *Saccharomyces cerevisiae*. It revealed that the highest concentrations of crude protein (20g/l) was produced in fruit hydrolysates media supplemented with glucose and nitrogen on the 16th day of fermentation as compared to the control fruit hydrolysates (5.6 g/l) without nutrient or glucose supplementation.

These observations highlighted the importance of supplementation to increase SCP production. The study of Akhilesh Bind *et al.* (2013) revealed that a combination of orange, banana, and papaya peels in

a ratio 1:2:1 showed fungal biomass of *Aspergillus niger* contained maximum protein content of 1.352 mg/ml at pH 7 on 8 days of incubation and maximum fungal growth than the other peel combinations²³.

A previous study also observed that the crude protein content of *A. niger* was 27.15% for orange peels (*Citrus sinensis*)⁵. Biniyam Yalemtesfa *et al.* (2010) also reported that *A. niger* (KA-06) exhibited a maximum protein yield of 35% from the fermentation of orange waste enriched with (NH₄)₂SO₄ as a nitrogen source with an inoculum load of 10⁶ spores/ml²⁴.

TABLE 2: EFFECT OF NUTRIENT SUPPLEMENTATION ON FUNGAL BIOMASS AND PROTEIN PRODUCTION

		Day	<i>A. oryzae</i>	<i>A. niger</i>
Mat weight (gm/l)	CMPM	D6	0.19±0.002	0.63±0.000
		D7	0.26±0.000	0.67±0.005
	SMPM	D8	0.32±0.001	0.71±0.005
		D9	0.35±0.005	0.76±0.002
		D10	0.40±0.004	0.84±0.004
		D11	0.46±0.060	0.92±0.002
		D6	0.31±0.000	0.65±0.005
	GMPM	D7	0.40±0.005	0.71±0.000
		D8	0.46±0.005	0.78±0.010
		D9	0.48±0.001	0.81±0.000
		D10	0.54±0.005	0.89±0.005
D11		0.61±0.007	1.22±0.001	
D6		1.79±0.005	2.29±0.005	
Protein (gm/l)	CMPM	D7	1.86±0.010	2.41±0.005
		D8	2.06±0.005	2.63±0.000
	SMPM	D9	2.17±0.005	3.18±0.005
		D10	2.28±0.005	3.34±0.003
		D11	2.48±0.080	3.53±0.005
		D6	1.05±0.000	2.24±0.000
		D7	1.09±0.000	2.28±0.050
	GMPM	D8	1.15±0.005	2.30±0.005
		D9	1.21±0.000	2.32±0.005
		D10	1.20±0.006	2.25±0.010
		D11	1.18±0.030	2.22±0.003
D6		1.19±0.005	2.59±0.005	
D7		1.21±0.005	2.64±0.050	
Protein (gm/l)	CMPM	D8	1.26±0.005	2.68±0.005
		D9	1.23±0.005	2.73±0.005
	SMPM	D10	1.21±0.005	2.65±0.007
		D11	1.76±0.001	2.62±0.004
		D6	1.80±0.050	2.66±0.000
		D7	1.98±0.005	2.71±0.005
		D8	2.06±0.005	2.82±0.010
	GMPM	D9	1.96±0.005	2.85±0.000
		D10	1.86±0.010	2.72±0.002
		D11	1.83±0.009	2.70±0.001

Samples were evaluated in triplicate and calculated as mean ±SD (n=3). [CMPM-Control Mandarin Peel Media, SMPM-Supplemented Mandarin Peel Media; GMPM-Glucose Supplemented Mandarin Peel Media].

Chemical Compositions (Carbohydrate, Fat, Fiber, Moisture, Ash) and Estimation of Total Genomic DNA Content of Fungal Biomass in Different Types of Mandarin Peel Media with or without supplementation at 9th Day of Fermentation: Table 3 showed that after 9th day of fermentation highest amount of total carbohydrate content of fungal biomass was recorded for *A. niger* in GMPM (1.34gm/l) while the least was from control mandarin peel medium with *Aspergillus oryzae* (0.71gm/l).

The results of Abarshiet *al.* (2017) also expressed that fruit hydrolysate medium supplemented with glucose and nitrogen yielded the highest concentration of total carbohydrate (6.6 gm/l) on the 16th day of fermentation than fruit hydrolysate media without any supplementation for the production of SCP.

The findings of A. Mondal *et al.*, (2012) reported that after fermentation highest amount of crude carbohydrate produced from orange peels and cucumber peels through solid-state fermentation, the resulting biomass of *Sachharomyces cerevisiae* was 39.66% and 50.5%, respectively in glucose-supplemented fruit hydrolysate media. The study of Kalpana C *et al.*, (2015) showed that after

bioconversion of fruit and vegetable waste could produce single-cell protein using *A. oryzae* and *Sachharomyces cerevisiae* ²⁵. In this study the highest amount of carbohydrate was found in fungal biomass of *A. oryzae* obtained from glucose supplemented medium than *Sachharomyces cerevisiae* of glucose-supplemented medium.

Maximum fat content was observed (1.24gm/l) in *A. niger* biomass produced GMPM medium at 9th day of fermentation, whereas biomass of *A. oryzae* produced in CMPM medium had the least amount of crude fat content (0.76%). Saquid Azam *et al.*, (2014) ⁵ also showed that after bioconversion of citrus peels, biomass of *A. niger* produced in glucose supplemented obtained fat content of 0.60%.

A. niger biomass produced in GMPM medium contained the highest crude fiber (1.55gm/l) content. The study conducted by Sadique Azam *et al.* (2014) showed that biomass obtained from citrus peels using *A. niger* had 2.40% crude fiber content. The moisture content of fungal biomass varied from 59.87±0.02-70.03±0.005%. The highest moisture content was observed in the biomass of *A. oryzae* in CMPM.

TABLE 3: CHEMICAL COMPOSITIONS (CARBOHYDRATE, FAT, FIBER, MOISTURE, ASH) AND ESTIMATION OF TOTAL GENOMIC DNA CONTENT OF FUNGAL BIOMASS IN DIFFERENT TYPES OF MANDARIN PEEL MEDIA WITH OR WITHOUT SUPPLEMENTATION AT 9th DAY OF FERMENTATION (DRY MATTER BASIS)

Carbohydrate (gm/l)		<i>A. oryzae</i>	<i>A. niger</i>
	CMPM	0.62±0.002	1.15±0.005
	SMPM	0.68±0.010	1.22±0.005
	GMPM	0.71±0.005	1.34±0.001
Fat (gm/l)	CMPM	0.76±0.010	1.03±0.050
	SMPM	0.92±0.004	1.16±0.005
	GMPM	1.08±0.002	1.24±0.000
Fibre (gm/l)	CMPM	0.67±0.003	1.45±0.010
	SMPM	0.78±0.004	1.50±0.003
	GMPM	0.84±0.003	1.55±0.005
Moisture (%)	CMPM	70.03±0.001	62.43±0.00
	SMPM	69.02±0.002	61.72±0.01
	GMPM	64.15±0.002	59.87±0.00
Ash (%)	CMPM	2.17±0.001	2.62±0.002
	SMPM	2.34±0.001	3.01±0.001
	GMPM	2.52±0.001	3.81±0.010
Total genomic DNA (%)	CMPM	1.55±0.00	1.91±0.002
	SMPM	1.67±0.003	2.09±0.001
	GMPM	1.80±0.001	2.20±0.000

Samples were evaluated in triplicate and calculated as mean ±SD (n=3). [CMPM-Control Mandarin Peel Media, SMPM-Supplemented Mandarin Peel Media; GMPM-Glucose Supplemented Mandarin Peel Media].

Maximum ash content was observed in *A. niger* biomass in GMPM (3.81%) where *A. oryzae* in CMPM medium released lowest amount of ash (2.17%). Azam et al. (2014) study revealed that the ash content of *A. niger* biomass using different orange peels in solid-state fermentation ranged from 4.00-4.8%. Foods with high nucleic acid content were reported as toxic for human consumption. SCP contained higher nucleic acid content, which is why they have not been used as human food. In this study, the total genomic DNA contents of different fungal biomasses ranged from 1.55±0.001-2.20±0.001% **Table 3**. The amount of genomic DNA was much lower than the genomic DNA content (6.60±0.25%) of *Candida utilis* biomass obtained from mango waste²⁶.

Effect of Nutrients Supplementation on Substrate Utilization Efficiency (%) of Fungi and Fungal Substrate Utilization Proportionality Constant (K_u g/l) in Different Types of Mandarin Peel Media with or Without Supplementation: **Table 4** expressed how much amount of fermentable sugar in the medium was utilized by the two fungi. The bioconversion of total carbohydrate was maximum (71.4±0.05%) by *A. niger* in glucose supplemented media due to the presence of simple sugars and fermentable sugars in GMPM. It was previously reported in the study of Kandari *et al.* (2012) that the conversion of fermentable sugars by *S. cerevisiae* from vegetable and fruit peel extract was very high, recorded as 94-98%¹⁷.

TABLE 4: EFFECT OF NUTRIENTS SUPPLEMENTATION ON SUBSTRATE UTILIZATION EFFICIENCY (%) OF FUNGI AND FUNGAL SUBSTRATE UTILIZATION PROPORTIONALITY CONSTANT (K_u G/L) IN DIFFERENT TYPES OF MANDARIN PEEL MEDIA WITH OR WITHOUT SUPPLEMENTATION

	<i>A. oryzae</i>	<i>A. niger</i>
Substrate utilization efficiency (%)		
CMPM	53.21±0.00	61.14±0.003
SMPM	60.10±0.002	69.45±0.002
GMPM	68.85±0.001	71.40±0.050
K_u g/l		
CMPM	0.86±0.003	1.57±0.003
SMPM	1.08±0.005	2.68±0.002
GMPM	1.45±0.010	2.93±0.050

Samples were evaluated in triplicate and calculated as mean ±SD (n=3). [CMPM-Control Mandarin Peel Media, SMPM-Supplemented Mandarin Peel Media; GMPM-Glucose Supplemented Mandarin Peel Media].

K_u value expressed how much substrate was utilized to produce fungal biomass. During bioconversion fungi used the fermentable sugar as carbon source and produced SCP. The highest K_u value (2.93±0.05g/l) was observed in GMPM medium by *A. niger*. Due higher carbohydrate content of Darjeeling mandarin peels and glucose supplementation, the K_u value increased in GMPM medium. It indicated that the sources of carbon, other nutrients, and glucose supplementation were maximum utilized by *A. niger* compared to *A. oryzae*. Lowest value of K_u was found in CMPM medium by *A. oryzae* (0.86±0.003g/l), which revealed that in this medium utilized less nutrients was utilized by the fungus and less biomass and SCP were produced.

CONCLUSIONS: The present findings revealed that fruit wastes like Darjeeling mandarin peels can be a potential substrate for producing SCP by submerged fermentation. The industrially important

fungi *Aspergillus niger* and *Aspergillus oryzae* were capable of converting fermentable carbohydrates into fungal biomass. Supplementation of nutrients into the fermentation media increased the fungal biomass and SCP production it was also observed that the addition of glucose as a carbon source into the fermentation media also enhanced the SCP production. This research work could minimize environmental pollution by using agro-wastes as inexpensive substrates for various bioconversion processes.

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CONFLICTS OF INTEREST: The authors are unanimous in publishing this paper. There is also nobody to contradict this manuscript.

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