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Research Article

Enhancement of alpha amylase production by *Aspergillus flavus* AUMC 11685 on mandarin (*Citrus reticulata*) peel using submerged fermentation

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ABSTRACT

Mandarin peel as submerged fermentation (SmF) source was tested for the production of alpha amylase enzyme by strain of *Aspergillus flavus* AUMC 11685. Incubation period, concentration of substrate, temperature, pH and size of inoculum were optimized to achieve the maximum production of alpha amylase enzyme by *Aspergillus flavus* using mandarin peel. The maximum production of alpha amylase enzyme by *Aspergillus flavus* was recorded at 4-5 days of incubation, 3% substrate concentration, inoculum concentration 10%, temperature 28-40°C and pH 4-5.5.

Keywords: Mandarin; α-amylase; *Aspergillus flavus*; Submerged fermentation.

1. INTRODUCTION

Nowadays, the new potential of using micro-organism as biotechnological source of industrially relevant enzymes has stimulated interest in exploration of extracellular enzymatic activities in several microorganisms [1-3]. Enzymes have been used for thousands of years to produce food and beverages, such as cheese, yoghurt, beer and wine [4].

Enzymes are protein catalysts synthesized by living systems and are important in synthetic as well as degradative process. Alpha amylase enzyme (α-1,4 glucan-glucanohydrolase) is widely distributed in nature. This extracellular starch degrading enzyme hydrolyses α-1,4 glucosidic linkages randomly throughout the starch molecule in an endo-fashion producing oligosaccharides and monosaccharides including maltose, glucose and alpha limit dextrin [5-8]. Alpha-amylase enzymes account 65% of enzyme market in world. Amylases had numerous applications including liquefaction of starch in the traditional beverages, baking and textile industry for desizing of fabrics [9-11]. Moreover, they have been applied in paper manufacture, medical fields as digestive and as detergent additives [12, 13]. Hence, any substantial reduction in the cost of production of enzymes will be a commercial positive stimulus [4]. Fungi are particularly interesting due to their easy cultivation, and high production of extracellular enzymes of large industrial potential. These enzymes have

commercial application in various industries [14].

Many useful enzymes are produced using industrial fermentation belonging to the genus *Aspergillus* [15, 16]. In fact *Aspergillus niger* is the largest fungal source of enzymes [17, 18]. α-amylase is widespread in animals, fungi, plants, and are also found in bacteria [19, 20]. Amylases from microbial sources are generally used in industrial processes due to a number of factors including productivity, thermostability of the enzyme as well as ease of cultivating microorganisms [21]. Alpha-amylases are produced commercially in bulk from microorganisms and represent about 25-33% of the world enzyme market [22].

Many attempts have been made to optimize culture conditions and suitable strains of fungi [23]. Selection of the microbial source for α-amylase production depends on several features, such as the type of culture (solid-state or submerged fermentation), pH and genotypic characteristic of the strain [24].

Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria and fungi. Several additional compounds also released apart from the usual products of fermentation called secondary metabolites which, range from several antibiotics to enzymes [25, 26]. The development of techniques such as Solid State Fermentation (SSF) and Submerged Fermentation (SmF) has lead to industrial-level production of useful enzymes. Submerged fermentation utilizes free flowing liquid substrates, such as broths, enzymes are secreted into the fermentation broth [27]. The purification of products is easier in SmF. More than 75% of the industrial enzymes are produced using SmF, one of the major reasons being that SmF supports the utilization of genetically modified organisms to a greater extent than SSF. Another reason why SmF is widely used is the lack of paraphernalia regarding the production of various enzymes using SSF. This is highly critical due to the fact that the metabolism exhibited by microorganisms is different in SSF and SmF [28]. Solid-state fermentation (SSF) has been defined as the fermentation process which involves solid matrix and is carried out in absence or near absence of free water. The solid matrix could be either the source of nutrients or simply a support supplemented by the suitable nutrients that allows the development of the microorganisms [29]. There are some disadvantages of SSF like difficulties on scale-up, low mix effectively, difficult control of process parameters (pH, heat, moisture, nutrient conditions), problems with heat build-up, higher impurity product and increasing recovery product costs [30]. Optimization of various parameters is one of the most important techniques used for the production of enzymes in large quantities to meet industrial demands [31]. Production of extracellular alpha-amylase in fungi is known to depend on the growth of mycelium and both morphological and metabolic state of the culture [32].

The selection of a substrate (agricultural waste) for enzyme production depends upon several factors mainly related with cost and availability of the substrate, the solid substrate not only supplies the nutrients to the microbial culture growing in it but also serves as anchorage for the cells [33]. These agriculture wastes consist of carbon and nitrogen sources necessary for the growth and metabolism of microorganisms [34, 35]. These nutrient sources included orange and mandarin wastes, rice and wheat bran, tea waste, cassava flour, oil palm waste, apple pomace and banana waste [36].

An increasing trend toward efficient utilization of natural resources has been observed around the world. The direct disposal of agro-industrial residues as a waste on the environment represents an important loss of biomass, which could be bioconverted into different metabolites, with a higher commercial value [37]. Citrus by-products are the principal solid by-product of the citrus processing industry and constitute about 50% of fresh fruit weight [38]. Mandarin considers as a source of multiple beneficial nutrients for human beings. Processing of citrus by-products potentially represents a rich source of phenolic compounds and dietary fibre. The mandarin peel wastes contribute the major industrial food waste discarded in the environment arising from juice manufacturing and home wastes [39]. Biotechnological applications of mandarin peel wastes are interesting not only from the point of view of low-cost substrate, but also in solving problems related to their disposal [40].

Although several investigations were employed on the production of enzymes by fungal strains using different agriculture wastes, only few

researches were done studying the production of enzymes by fungal strains using mandarin peel wastes. This work aims to evaluate the potentials of *Aspergillus flavus* strain AUMC 11685 isolated from accumulated rains water at Jeddah region to produce extracellular alpha amylase enzyme using mandarin peel wastes as substrate by submerged fermentation. Moreover, several factors including: pH, temperature, incubation period and concentration of each of raw material and inoculum were tested for optimization and enhancement of α-amylase enzyme production by *Aspergillus flavus* AUMC 11685 using mandarin peel wastes as a substrate in the submerged fermentation process.

2. MATERIALS AND METHODS

2.1. Microorganism

Pure culture of *Aspergillus flavus* AUMC 11685, which was isolated from accumulated rains water, Jeddah, Saudi Arabia, was grown and maintained on potato dextrose agar and it used as an inoculum during optimization steps of the study. The identification of the tested fungal species was confirmed by Assiut University Mycological Centre (AUMC) and the strain is deposited at Assiut University Mycological Centre under the code *Aspergillus flavus* AUMC 11685. The slants of the strain were grown at 28°C for seven days and stored at 4°C.

2.2. Agriculture wastes

Five grams of the agricultural waste; mandarin peel were mixed in 500 ml Erlenmeyer conical flasks containing 100 ml distilled water and sterilized in autoclave at 121°C for 20 min. Mandarin peel chosen as the sole nutrient source for submerged fermentation (SmF).

2.3. Optimization methodology of submerged fermentation (SmF)

Submerged fermentation was performed to study the effect of various physico-chemical parameters required for the optimum production of α-amylase enzyme by *A. flavus* AUMC 11685. Conidia are scrapped from mycelia of the terrestrial fungal species which are grown on slants for five days at 28°C and suspended in sterile distilled water. One ml of this suspension is used to inoculate, under aseptic conditions, Erlenmeyer flasks (500 ml capacity) each containing 100 ml of previous sterilized medium (agriculture waste medium). The inoculated flasks are incubated at 28°C on a rotary shaker at 160 rpm for 7 days (Figure 1). *Aspergillus flavus* was subjected to several optimization factors for enhancement of α-amylase enzyme production using mandarin peel wastes by SmF. Each experiment was done in thrice.

Figure 1. The inoculated flask containing the submerged fermentation medium of mandarin peel wastes.

2.3.1. Initial pH

The tested fungal strain of *Aspergillus flavus* was grown on mandarin peel medium by applying the previously mentioned fermentation process at different initial pH 2, 4, 5.5, 7 and 10. The initial pH was adjusted by 0.1 M HCl or 0.1 M NaOH. The assay of α-amylase produced was determined.

2.3.2. Incubation temperature

The tested fungal strain of *Aspergillus flavus* was grown on mandarin peel medium by applying the previously mentioned fermentation process at different incubation temperature degrees 20, 25, 28, 35, 40 and 50°C at the optimum initial pH. The assay of α-amylase produced was determined.

2.3.3. Incubation period

The tested fungal strain of *Aspergillus flavus*

was grown on mandarin peel medium by applying the previously mentioned fermentation process at several intervals of inoculation periods 2, 3, 4, 5, 6 and 7 days at both the optimum temperature and initial pH. The assay of α-amylase produced was determined.

2.3.4. Concentration of raw material

The tested fungal strain of *Aspergillus flavus* was grown on mandarin peel medium by applying the previously mentioned fermentation process at different concentration of raw material of mandarin peel waste 1, 3, 5, 7 and 9 g at the optimum temperature, initial pH and the optimal incubation period. The assay of α-amylase produced was determined.

2.3.5. Concentration of inoculum

The tested fungal strain of *Aspergillus flavus* was grown on mandarin peel medium by applying the previously mentioned fermentation process at different inoculum concentrations 0.5, 1, 2, 5 and 10 ml at the optimum temperature, initial pH, the optimal incubation period and raw material concentration. The assay of α-amylase produced was determined.

2.4. Partially purification of enzymes

Conical flasks containing the agriculture waste medium and the fungal inocula are filtered at the end of the incubation period. Then, the filtrate introduced into dialysis bag against distilled water for 24 hours. The dialyzed filtrate was centrifuged at 10,000 rpm for 20 min. The supernatant was pooled and designated as cell-free broth. The cell free broth was frozen at -20°C for further purification steps [41].

2.5. Enzyme assay

α-amylase activity was determined by measurement of glucose released from starch according to the method of Miller [42]. The reaction mixture in tubes contained 125 µl soluble potato starch 0.2%, 125 µl sodium acetate buffer, pH 5.5, 50 µl of enzyme solution and distilled water to give a final volume of 0.5 ml (test solution) and was incubated at 37°C for 30 min. The reaction was stopped by the addition of 0.5 ml dinitrosalicylic acid reagent (DNS), followed by incubation in a boiling water bath for 10 min followed by cooling. The absorbance was recorded at 560 nm. The enzymatically liberated reducing sugar was calculated from a standard curve using glucose. One unit of enzyme activity was defined as the amount of enzyme producing 1 µmol reducing sugar as glucose per minute under the standard assay conditions.

3. RESULTS

Alpha-amylase production by *Aspergillus flavus* AUMC 11685 isolated from water habitats in Jeddah, Saudi Arabia using mandarin peel by submerged fermentation was optimized.

3.1. The effect of pH

The result of the effect of different pH values on the production of α-amylase by *Aspergillus flavus* AUMC 11685 was shown in Table 1. The lowest productivity was obtained at pH 2 (7.32 U/ml), then the α-amylase activity sharply increased at pH 4 (24.73 U/ml), and gradually increased at pH 5.5 (26.90 U/ml). At pH values higher than 5.5 the productivity sharply decreased at pH 7 (17.99 U/ml) and at alkaline pH 10 (17.03 U/ml). The highest α-amylase enzyme production was recorded at pH 5.5.

3.2. The effect of incubation temperature

The result of the effect of different incubation temperature on the production of α-amylase was shown in Table 2. *Aspergillus flavus* has ability to produce α-amylase enzyme when incubated at temperature 20°C (15.76 U/ml) and 25°C (18.24 U/ml). α-amylase productivity sharply increased and recorded the highest productivity at 28°C (26.90 U/ml), then sharply inversed at 35°C (18.36 U/ml) and then declined gradually at 40°C (18.67 U/ml) and 50°C (14.38 U/ml). There was no noticeable change in amount of produced enzyme at temperature; 25, 35 and 40°C.

Table 1. Effect of different pH values on α-amylase production (U/ml) by *Aspergillus flavus* isolated from water habitats in Saudi Arabia using mandarin peel wastes as submerged culture.

pH values	Extracellular α-amylase production (U/ml)
2	7.32
4	24.73
5.5	26.90
7	17.99
10	17.03

One unit of α-amylase enzyme activity was defined as the amount of enzyme producing 1 µmol reducing sugar as glucose per minute under the standard assay conditions.

Table 2. Effect of different incubation temperatures on α-amylase production (U/ml) by *Aspergillus flavus* isolated from water habitats in Saudi Arabia using mandarin peel wastes as submerged culture.

Incubation temperatures	Extracellular α-amylase production (U/ml)
20 ºC	15.76
25 ºC	18.24
28 ºC	26.90
35 ºC	18.36
40 ºC	18.67
50 ºC	14.38

One unit of α-amylase enzyme activity was defined as the amount of enzyme producing 1 µmol reducing sugar as glucose per minute under the standard assay conditions.

3.3. The effect of different concentrations of substrate (mandarin peel)

The result of the effect of different concentrations of mandarin peel medium on the production of α-amylase was shown in Table 3. Our results showed that *A. flavus* could produce small amount of α-amylase using mandarin peel medium at concentration 1% (g/100 ml) (12.82 U/ml), then pointedly increased to the highest yield at concentration 3% (28.28 U/ml) and slightly decreased at concentration 5% (26.90 U/ml). After this, the productivity decreased gradually at concentrations 7% (17.24 U/ml) and 9% (16.79 U/ml).

Table 3. Effect of different concentrations of mandarin peel medium on α-amylase production (U/ml) by *Aspergillus flavus* isolated from water habitats in Saudi Arabia using mandarin peel wastes as submerged culture.

Concentration of mandarin peel medium	Extracellular α-amylase production (U/ml)
1 g	12.82
3 g	28.28
5 g	26.90
7 g	17.24
9 g	16.79

One unit of α-amylase enzyme activity was defined as the amount of enzyme producing 1 µmol reducing sugar as glucose per minute under the standard assay conditions.

3.4. The effect of incubation period

Alpha-amylase production was detected at different incubation periods as shown in Table 4. *Aspergillus flavus* could start α-amylase production using mandarin peel medium after two days of incubation (13.46 U/ml) and then the productivity increased in gradual trend at three days of incubation (18.10 U/ml). α-amylase production sharply increased recording the peak rate at the fourth day of incubation (33.52 U/ml), then progressively decreased in gradual trend at five (28.93 U/ml), six (27.12 U/ml) and seven (26.90 U/ml) days of incubation. The highest α-amylase enzyme production was obtained after incubation for 4 days.

3.5. The effect of inoculum concentration

The result of the effect of different concentrations of *A. flavus* inoculum on the production of α-amylase was displayed in Table 5. Little output of α-amylase was detected by inoculum concentration 0.5% of *A. flavus* (3.04 U/ml), sharply increased by inoculum concentration of 1% *A. flavus* (26.90 U/ml). α-amylase productivity soared gradually by inoculum concentration 2% of *A. flavus* (30.04 U/ml) and by inoculum concentration 5% of *A. flavus* (35.73 U/ml), then it boosted the highest significant increment by inoculum concentration 10% of *A. flavus* (64.30 U/ml).

Table 4. Effect of different Incubation periods on α-amylase production (U/ml) by *Aspergillus flavus* isolated from water habitats in Saudi Arabia using mandarin peel wastes as submerged culture.

Incubation periods	Extracellular α-amylase production (U/ml)
2 days	13.46
3 days	18.10
4 days	33.52
5 days	28.93
6 days	27.12
7 days	26.90

One unit of α-amylase enzyme activity was defined as the amount of enzyme producing 1 µmol reducing sugar as glucose per minute under the standard assay conditions.

Table 5. Effect of different Inoculum concentrations on α-amylase production (U/ml) by *Aspergillus flavus* isolated from water habitats in Saudi Arabia using mandarin peel wastes as submerged culture.

Inoculum concentration	Extracellular α-amylase production (U/ml)
0.5 ml	3.04
1 ml	26.90
2 ml	30.04
5 ml	35.73
10 ml	64.30

One unit of α-amylase enzyme activity was defined as the amount of enzyme producing 1 µmol reducing sugar as glucose per minute under the standard assay conditions.

4. DISCUSSION

The production of α-amylase using submerged fermentation by fungi has been reported by many workers [43-46]. In the present study, the optimum conditions for α-amylase production by *Aspergillus flavus* were acidic pH range 4-5.5, a temperature of 25-40°C for a period of 4-5 days using concentration of mandarin peels medium 3-5% and the concentration of *A. flavus* microbial suspension was positively related with productivity.

From our results extracellular α-amylase could be produced by *A. flavus* using mandarin peels at all pH values used but with different amounts. Extreme pH values (highly alkaline or acidic) decreased α-amylase production. At temperature 28°C, *A. flavus* showed the maximum α-amylase production, whereas below or above this temperature α-amylase production declined gradually. Extracellular α-amylase could be produced by *A. flavus* using mandarin peels (concentration 1%) and increased at concentration 3%, above this concentration there was a negative relation between α-amylase productivity and concentration of mandarin peels medium. After 4 incubation days *A. flavus* showed the maximum α-amylase production, whereas at less than this the α-amylase production declined or more than 4 days the productivity declined gradually. There was positive relation between concentration of *A. flavus* microbial suspension and α-amylase production. Our study reported that the highest α-amylase enzyme production by *A. flavus* isolated from water habitats in Saudi Arabia using mandarin peels medium was recorded at pH 5.5, temperature 28°C and incubation period of 4 days. The maximum productivity of α-amylase was detected when using concentration 3 g/100 ml of mandarin peels medium and 10% concentration of *A. flavus* microbial suspension.

Among the physical parameters, the pH of medium plays an important role by inducing morphological changes in fungi and in enzyme secretion [47]. The synthesis of extracellular α-amylase is affected by the pH [48].

In agreement to our results, Sivaramakrishnan et al. [49] who reported that alpha amylase enzyme synthesis occurred at pH range 3-9 with an optimum at pH 5 by *Aspergillus oryzae* on wheat bran. Our results are also nearly similar to those obtained by Acourene et al. [47] who reported that a maximum biomass was produced at pH=6.0, and the lowest at pH=9.0 and pH=4.0 during their study on alpha amylase production by *Candida guilliermondii* on date wastes. Also more or less similar findings confirmed by Djekrif-Dakhmouche et al. [34], Hernandez et al. [43], Alva et al. [50] and Renato and Nelson [51] on *Aspergillus* spp., Silva et al. [52] on *Penicillium purpurogenum* and *A. niger* at pH varying between 5.0 and 6.0. Guillen-Moreira et al. [53], reported that the growth and α-amylase enzyme production by *Aspergillus tamarii* were inhibited when the initial pH of the medium was above 10.0 or below 4.0. In contrast, Pavezzi et

al. [54] reported that pH=4.0 to be the best for the production of α-amylase by *A. awamori*. With inconsistence of our results Suganyadevi et al. [55] reported that the maximum production of α-amylase by *A. niger* on tuber of *Ipomoea batatas* was attained at pH 7. Moreover, Varalakshmi et al. [56] and Arunsasi et al. [8] found that the highest production of α-amylase by *Aspergillus flavus* on wheat bran and *Cocos nucifera* meal was accomplished at pH 7.5.

Temperature is one of the important factors, which strongly affect alpha amylase production by fermentation process [19, 57, 58]. Our findings were compatible with Suganyadevi et al. [55] who observed that the maximum yield of α-amylase production by *A. niger* was possible by submerged fermentation supplied with tuber of *Ipomoea batatas* at room temperature (28°C). Our results are also similar to those obtained by Ramachandran et al. [59] who studied α-amylase enzyme synthesis by *Aspergillus oryzae* on coconut oil cake and reported that 30°C proved to be the best temperature for the enzyme synthesis. In addition, similar results were obtained by Arunsasi et al. [8] who studied α-amylase enzyme production by *Aspergillus flavus* on *Cocos nucifera* meal.

Incubation at higher temperature affected the fungus harmfully. In agreement of our output Sivaramakrishnan et al. [49] reported that alpha amylase enzyme synthesis by *Aspergillus oryzae* occurred between 20-45°C with an optimum at 30°C on wheat bran. Acourene et al. [47] reported that alpha-amylase production by *Candida guilliermondii* on date wastes was low at 20°C, and increased to a maximum at 30°C. A further increment in temperature resulted in a decrease in dry biomass and α-amylase production. At higher temperature, due to the production of large amount of metabolic heat, the fermenting substrate temperature shoots up, thereby inhibiting microbial growth and enzyme formation [60]. Temperature above 45°C results in moisture loss of the substrate, which affects metabolic activities of fungi, and results in reduced growth and α-amylase production [61]. However, Kunameni et al. [62] and Ravi et al. [63] reported that optimum temperature for amylase production by *Trichoderma lanuginosus* and *Humicola lanuginosa* is 50°C. Moreover, the optimum temperature for the maximum α-amylase activity by some *Aspergillus* spp. was 30°C [34, 45, 46, 50, 51] and also the same by *Penicillium brevicompactum* [64] and *Penicillium purpurogenum* [52].

Regarding the impact of incubation period on alpha amylase production, our findings were nearly came in agreement with Kareem et al. [36] who reported that the maximum α-amylase production by *Aspergillus oryzae* on *Cowpea* wastes was recorded after 72 hours of incubation. Sivaramakrishnan et al. [49] also reported the same during on wheat bran and Acourene et al. [47] with *Candida guilliermondii* on date wastes. In contrast to our results, Silva et al. [52] observed the highest production by *Penicillium purpurogenum* and *Penicillium brevicompactum* after 6 and 7 days of incubation and Balkan and Ertan [64] after 7 days with *Penicillium brevicompactum*.

No doubt that concentration of substrate affects α-amylase production. Similar to our findings Mohamed et al. [41] who studied the effect of mandarin peel concentration on α-amylase production by *Trichoderma harzianum* found that the highest level of enzyme activity was obtained at 5% of mandarin peel. Further concentration of mandarin peel repressed the enzyme production. Ramachandran et al. [59] reported that 0.5% concentration of starch was most suitable and higher concentrations of starch resulted in the inhibition of α-amylase enzyme synthesis by *Aspergillus oryzae* (data not shown).

The inoculum concentration has been reported as an important factor in enzymes production by fermentation. Lower inoculum concentration required longer time for the cells to multiply to sufficient number to utilize the substrate and produce enzyme. An increase in the number of spores in inoculum would ensure a rapid proliferation and biomass synthesis. Ramachandran et al. [59] reported that enzyme production increased with the increase in inoculum size from the lowest value of 0.5 ml and this in agreement of our current study, and they also reported that the maximum enzyme activity at 2 ml inoculum, further increase in the inoculum size resulted in decreased enzyme synthesis, indicating that limitation of nutrients occurred due to the increased microbial activity (results not shown) but this is not compatible with our results. Balkan and Ertan [64] reported that inoculum concentration 2.5 ml of *Penicillium brevicompactum*

gave the maximum production of alpha-amylase. Kareem et al. [36] reported that the maximum amylase production of α-amylase enzyme is attained at 4% *Aspergillus oryzae* inoculum level on *Cowpea* wastes and a further increase in the inoculums size did not increase the amylase yield. A lower level of inoculum may not be sufficient for initiating growth and enzyme synthesis.

General outlook indicates that our results are promising in enhancement of alpha-amylase production by growing strain of *Aspergillus flavus* AUMC 11685 on mandarin peel wastes in submerged culture fermentation. Based on the results obtained, mandarin peel wastes and our strain of *Apergillus flavus* were nearly more efficient in the quantity of alpha amylase production at the optimal conditions when they were compared with other wastes or substrates and microorganism in reported previous works. We have obtained 64.30 U/ml whereas Balkan and Ertan [64] detected 40 U/ml on rye straw, 50 U/ml on wheat straw, 25 U/ml on wheat branand 160 U/ml on corncob leaf by *Penicillium chrysogenum*, Farid and Shata [65] detected 1362.09 IU/g on wheat flour by *Aspergillus oryzae* LS1, Acourene et al. [47] estimated 1519.23 µmol/l/min on date wastes by *Candida guilliermondii* CGL-A10, Hang and Woodams [66] harvested 29 U/ml on baked-bean wastes and 0.06 U/ml on 2% cornmeal by *Aspergillus foetidus* NRRL 337, Suganthi et al. [67] found 43 U/mg on groundnut oil cake by *Aspergillus niger* BAN 3E, Singh et al. [27] indicated 11.0 U/ml on bacteriological peptone, $MgSO_4 \cdot 7H_2O$, KCl, starch by *Bacillus* sp., Krishna et al. [68] evaluated 23 U/ml on banana peel by *Aspergillus niger* NCIM 616 and Kumar et al. [69] produced 90 U/ml on sweet lime peel by *Aspergillus niger*.

5. CONCLUSION

The present study reveals that mandarin peel waste can be used safely as optional substrates than other agricultural/agro-industrial wastes such as wheat, corn, rice, potato and apple for the production of α-amylase enzyme. This study established the potential of the fungal strain of *Aspergillus flavus* AUMC 11685 for economic α-amylase production on mandarin peel in optimum conditions. This work gives an insight into the exploitation of a new agriculture wastes for the production of some industrial enzymes in appreciable levels.

AUTHORS' CONTRIBUTION

All the authors contributed in the success of this research article. The final manuscript has been prepared and revised by EHA and AMR. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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