ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Enhanced tannase production by *Kluyveromyces marxianus* NRRL Y-8281 under solid state fermentation of olive oil cake.

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ABSTRACT

Valorization of agro-industrial wastes receives great attention in recent years. The production of olive oil is associated with the accumulation of vast amount of a solid waste known as olive cake (OC) that is difficult to be disposed. OC represents a huge environmental problem for olive oil producing countries. The present study was conducted for the possible utilization of OC for tannase production under solid-state fermentation (SSF) in a trial for its valorization. Of the ten strains tested, the yeast *Kluyveromyces marxianus* NRRL Y-8281 showed the highest tannase production capacity. Different fermentation parameters were studied for optimization of production process. Tannase yield was enhanced 1.40 fold as compared to un-optimized condition (1714.7 U/ g dry substrate, 1226.6 U/ g dry substrate, respectively) by growing the yeast at 45°C with initial pH 6.0, 20 % inoculum size and an initial moisture OC level of 35% for 48 hours. No carbon source or nitrogen source was required for enhancing the enzyme yield. Yeast is less explored as a source for tannase enzyme production compared to other microorganisms, however, it is a promising source for tannase production.

Keywords: Olive cake, solid state fermentation, tannase, yeast.

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ISSN: 0975-8585

INTRODUCTION

In olive oil industrial sectors, two approaches are known for olive oil extraction namely, the traditional discontinuous pressing system and the continuous centrifugation process. The latter is sub-distinguished into three phase decanter system and two phase decanter system. Two major byproducts are generated from the three phase decanter system, a solid residue (30%) known as olive cake (OC) which consists of olive skin, pulp and pit in addition to significant residual oil content and a liquid waste (50%) known as olive mill waste water [1]. About 30-35% of the total olive fruit weight is discarded as OC during olive oil extraction using three phase decanter system [2]. The worldwide production of OC is estimated to be 2,881,500 tonnes/ year [3]. The large amount of OC pumped into the environment over a very short time period (3-4 month representing olive harvest and pressing season) together with its unfavorable characteristics (high organic load, phenolic content and acidity and resistance to biological degradation) and the rapidly growing olive industries create serious seasonal environmental problems and a challenging disposal task for olive oil producing countries regarding both environmental and economic perspectives [4].

Tannin acyl hydrolase (E.C 3.1.1.20) (also known as tannase) is an intracellular or extracellular enzyme capable of hydrolyzing the ester bond of hydrolysable tannin, ellagitannin or gallotannin producing gallic acid, glucose and galloyl esters [5]. The major commercial applications of tannase reside in food, feed, beverages, pharmaceutical and chemical industries with the production of gallic acid [6].

From the industrial point of view, microbial tannases are more preferred than those derived from plants or animals as they express higher stability and their purification is much easier. In addition, microorganisms can produce large amount of tannase enzyme over a short time period via fermentation [7].

Most of the research was focused on fungal tannases. Besides being highly resistant, fungal tannses are active over a wide range of pH and temperature [8]. However the slow degradation provided by fungi as well as the difficulty of their genetic manipulation limit fungi utilization for industrial applications [9]. On the other hand, many tannin degrading bacteria have been isolated, however, their utilization at the industrial level is limited due to high cost of maintenance at production level [10].

Recently, much attention has been paid on the production of tannase from yeast sources due to its shorter generation time and similarity with fungal tannases. The ease of yeast cultivation on simple defined media is an added advantage for yeast tannase. Moreover, cultivation of yeast strains is well studied at industrial scales so standardization of fermentation process parameters for large scale production may not be an obstacle. Though studies on yeast tannase are scarce, yeast is a promising source for tannase production due to its high biotechnological potential [8].

SSF has attracted more attention as an alternative tannase production technique mainly due to the advantages provided by SSF over submerged fermentation (SmF) [11]. If compared to SmF, SSF is much simpler and cost effective [12]. Besides, SSF consumes less energy and water than SmF [13]. On the other hand, tannases produced through SSF show higher activity, higher productivity and higher stability to a wide range of pH and temperature comparing to those produced by SmF [14, 15]. To the best of our knowledge OC was not previously used as a solid support for tannase production by SSF technique, however the enzyme has been produced using several different agro-industrial wastes as solid supports.

The present work aims to use OC, which is considered as an environmental pollutant, as a cheap substrate for production of microbial tannase which has potent industrial applications and also to study the influence of fermentation parameters on enhancing the production of the enzyme.

MATERIALS AND METHODS

Olive oil cake waste:

OC was provided during its harvesting season by a local olive-pressing factory (three phase decanter system), located in Al-Arish, North Sinai, Sinai Peninsula, Egypt. It was stored at 4°C till used.

Microorganisms:

The bacterial strains (*Bacillus amyloliquifaciens* NRRL B-14393, *Bacillus subtilis* NRRL B-4219) and yeast strains (*Kluyveromyces marxianus* NRRL Y-8281, *Kluyveromyces marxianus* NRRL Y-7571, *Saccharomyces cerevisiae* NRRL Y-12632, *Candida bambicola* NRRL Y-17069, *Candida guilliermondii* NRRL Y-2075) used in this study were obtained from Agricultural Research Service, Peoria, Illinois, USA. While the fungal strains (*Agaricus blazei, Ganoderma lucidum, Hericium erinaceus*) were obtained from Central Laboratory for Agricultural Climate, Dokki, Giza, Egypt.

Culture maintenance and inoculum preparation:

Adaptation and inoculum preparation of yeast and bacterial strains

The yeast strains were streaked on YME medium with agar [16] for 48 h at 30°C, while the bacterial strains were streaked on a nutrient agar slants and incubated for 24 h at 37°C. All stock cultures were stored at 4°C and subculture every 4 weeks, then stored at 4°C. A loop of each culture was inoculated in 50 ml of sterile inoculum medium (composed of the stock medium without agar), then incubated on controlled incubator shaker (New Brunswick Scientific, USA) at 150 rpm for 24h at 30°C and 37°C, respectively. For SSF, an aliquot of 1 mL of each inoculum was inoculated in 250 ml Erlenmeyer flasks containing 5g of sterilized OC (sterilized at 121°C for 20 min. at 15 psi). Incubation was done at static incubator for 48 and 24 h at 30°C and 37°C for yeast and bacteria, respectively.

Adaptation and inoculum preparation of fungal strains

The fungal cultures were maintained by growing the fungal strains on stock slant medium (PDA) [17] for 1 week at 28-30°C. All stock cultures were stored at 4°C and subculture every 4 weeks, then stored at 4°C. One cm^3 of mycelium was transferred under sterilized conditions to 250 ml Erlenmeyer flasks containing 5 g of sterilized OC. Incubation was done at static incubator for 3 days at 30°C.

Enzyme extraction:

The enzyme was extracted from the fermented medium with 8-fold (v/w) acetate buffer (0.02 M, pH 5.5) by shaking (200 rpm) at 30ºC for 60 min. The resultant slurry was centrifuged at 10,070 xg for 15 min. at 4°C. Finally, the extracts were collected and considered as a source of crude enzyme.

Enzyme assay:

Tannase activity in culture supernatant was determined spectrophotometrically by the method of Ibuchi *et al.* [18]. To four parts of substrate (0.350 w/v % of tannic acid dissolved in 0.05 M citrate buffer, pH 5.5), one part of the enzyme solution was added. After (t) minutes reaction at 30°C, 0.1 part of the mixture was added to ten parts of 90% ethanol. The optical density of the ethanol solution at 310 nm was measured. Tannase activity (unit/ml) was given by following equation.

$$U = 114 \times \frac{E_{t1} - E_{t2}}{t2 - t1}$$

Where E_{t1} and E_{t2} mean the optical density of the ethanol solution at 310 nm prepared after t_1 and $t_2$minutes reaction. The enzyme activity was expressed in unit per gram dry substrate (U/gds), where one unit of the enzyme means the amount of the enzyme which is able to hydrolyze one micromole of the ester bond in tannic acid in one minute.

Optimization of Process Parameters:

Various physico-chemical and nutritional parameters influencing enzyme production during solid state fermentation were optimized. The protocol adopted for optimization of various process parameters was to evaluate the effect of an individual parameter (incubation time, carbon source, nitrogen source, etc…) independent of the others and subsequently optimal condition was incorporated in the experiment for

optimizing the next parameter. All experiments were carried out in triplicate and the mean values were reported.

Effect of incubation period:

After inoculation with 1 ml inoculum, the flasks were incubated at 30°C and the enzyme activity was measured after different time periods ranging from 6h to 96h.

Effect of different carbon sources:

To study the effect of supplementation of additional carbon sources at 1% (V/W) concentration on the production of the enzyme, some carbon sources were used in the fermentation medium namely glucose, galactose, fructose, mannose, sucrose, lactose, sorbitol, mannitol, starch, tannic acid, gallic acid and methyl gallate.

Effect of different nitrogen sources

Various inorganic (sodium nitrate, ammonium nitrate and ammonium chloride) and organic nitrogen sources (peptone, yeast extract, malt extract and urea) with a concentration of (1% W/W) were examined individually in the fermentation medium.

Effect of supplemented salt solution nutrient media:

The fermentation medium was moistened with 5 ml of salt solution before autoclaving. The composition of the salt solution was (W/V) 0.5% NH_4NO_3, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.1% NaCl at pH 5.5.

Effect of initial pH of the medium:

To study the effect of initial pH of the medium on tannase production, the initial pH was adjusted within a range from 3.0 to 9.0 with 1 N HCl or 1 N NaOH.

Effect of incubation temperature:

To study the effect of temperature on tannase production, the flasks were incubated at different temperatures ranging from 25 to 50°C.

Effect of inoculum size:

The effect of inoculum size on the production of tannase was investigated by growing the selected organism on the optimum culture medium conditions using different volumes of inoculum (1-30% v/w).

Effect of moisture content:

The effect of moisture content on tannase production was tested by varying the moisture content in the range of zero to 60%.

RESULTS AND DISCUSSION

In preliminary experiments, the capacity of 7 microorganisms to utilize OC as a sole carbon source and produce tannase enzyme was tested and shown in Table (1). Comparing these organisms with 3 strains which are known as potent tannase producers *Candida guilliermondii [19]*, *Saccharomyces cerevisiae* [13] and *Bacillus subtilis [14]*.

According to the data shown in Table (1), the yeast strain *Kluyveromyces marxianus* NRRL Y-8281 was the most potent strain for tannase production using OC as substrate for SSF compared to the other organisms giving 1226.6 U/g dry substrate so it was selected for completing this study.

ISSN: 0975-8585

Table (1): Tannase activity of ten microbial strains cultivated on raw OC for 48 hours of incubation at 30°C

Strain	Enzyme activity (U/gds)
*Unfermented olive cake	0
Bacteria:	
Bacillus amyloliquifaciens NRRL B-14393	0
Bacillus subtilis NRRL B-4219	0.876
Yeast:	
***Kluyveromyces marxianus* NRRL Y-8281**	**1226.6**
Kluyveromyces marxianus NRRL Y-7571	288.25
Saccharomyces cerevisiae NRRL Y-12632	469.06
Candida bambicola NRRL Y-17069	628.28
Candida guilliermondii NRRL Y-2075	436.16
Fungi:	
Agaricus blazei	0
Ganoderma lucidum	0
Hericium erinaceus	0

* Unfermented olive cake: non-cultivated autoclaved OC.

The optimum fermentation conditions and regulatory mechanisms for tannase enzyme production vary significantly among different microorganisms and generalization therefore is not possible [7].

Data in Table (2) showed that the use of the most common salt solution media for tannase production as the moistening media in the fermentation process resulted in a decrease in the enzyme production from 1226.6 U/gds to 1096.6 U/gds. The media consisted of (W/V) 0.5% NH_4NO_3, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.1% NaCl, pH 5.0 [20-23]. So, raw OC without added nutrient media was chosen for further studying.

Table (2): Effect of common salt solution nutrient media on production of *K. marxianus* NRRL Y-8281 tannase

Sample	Enzyme activity (U/gds)
OC without additive media	**1226.6**
OC with salt solution media	1096.6

-Fermentation was carried

The incubation period seems to have a significant influence on the enzyme production. Forty eight hours of fermentation was found to be the optimum incubation time giving maximum enzyme activity of 1226.6 U/gds (equivalent to 94.54 U/ml) after which the enzyme level starts to fall as shown in Fig.(1). It is stated that tannase is primarily produced during the primary phase of microbial growth [24], and the decrease in enzyme production after the optimum incubation period may be due to the start of the declining phase of the microorganism [25]. Decrease in enzyme production after the optimum incubation time may also be due to competitive inhibition of tannase enzyme by its accumulating product gallic acid [13], enzyme denaturation and/or degradation [26], decreased nutrient level of the media [27] or prolonged microbial cells incubation in the acidic media resulted from both enzyme substrate tannic acid and accumulated enzyme product gallic acid that can lead to either cell death or enzyme biosynthesis retardation [14].

Forty eight hours of fermentation was reported as optimum incubation time that insured maximum tannase production (33.1 U/ml) using a mixture of powdered fruits of *Terminalia chebula* and powdered pod cover of *Caesalpinia digyna* as solid support for fermentation with a co-culture of the *Rhizopus oryzae* and *Aspergillus foetidus* [28]. While, 96h of incubation was stated as optimum incubation time for fermentation of jamun leaves using *A. ruber [29]*.

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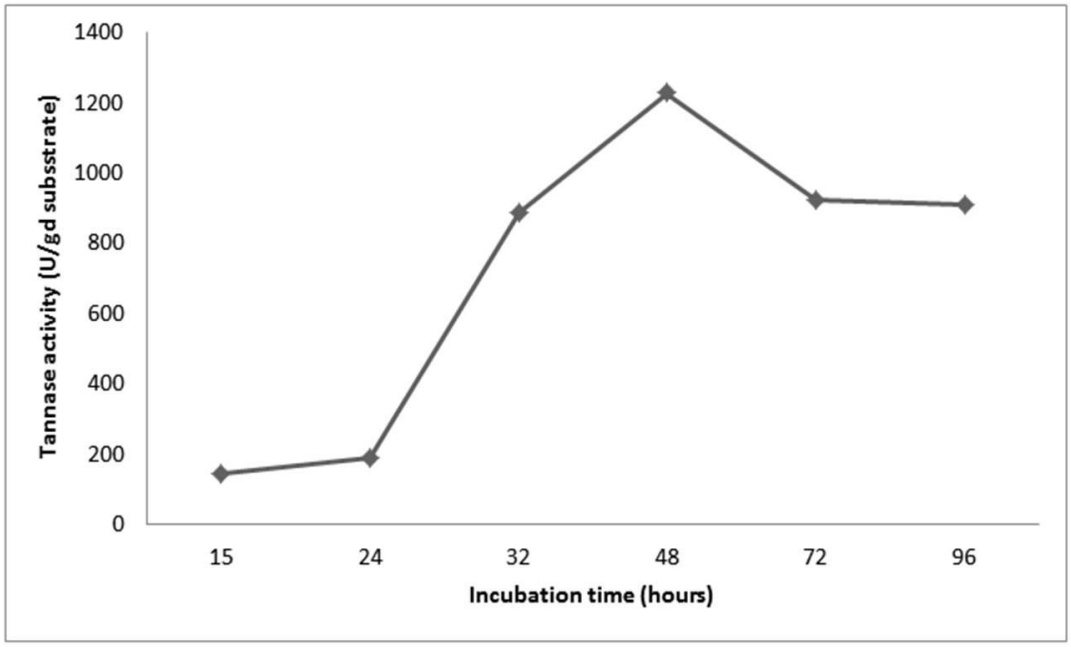

Fig. (1): Effect of incubation period on production of *K. marxianus* NRRL Y-8281 tannase

Available reports on the role of carbon sources on the extracellular secretion of tannase are contradictory. All the added carbon sources resulted in inhibition of *K. marxianus* tannase production by different degrees ranging from 7.2 to 91% as shown in Fig. (2). There are many reasons that can reasonably explain such behavior. The most important explanation is that the solid support medium used maybe already rich enough to supply the carbon source required for microbial growth and tannase enzyme production [29].

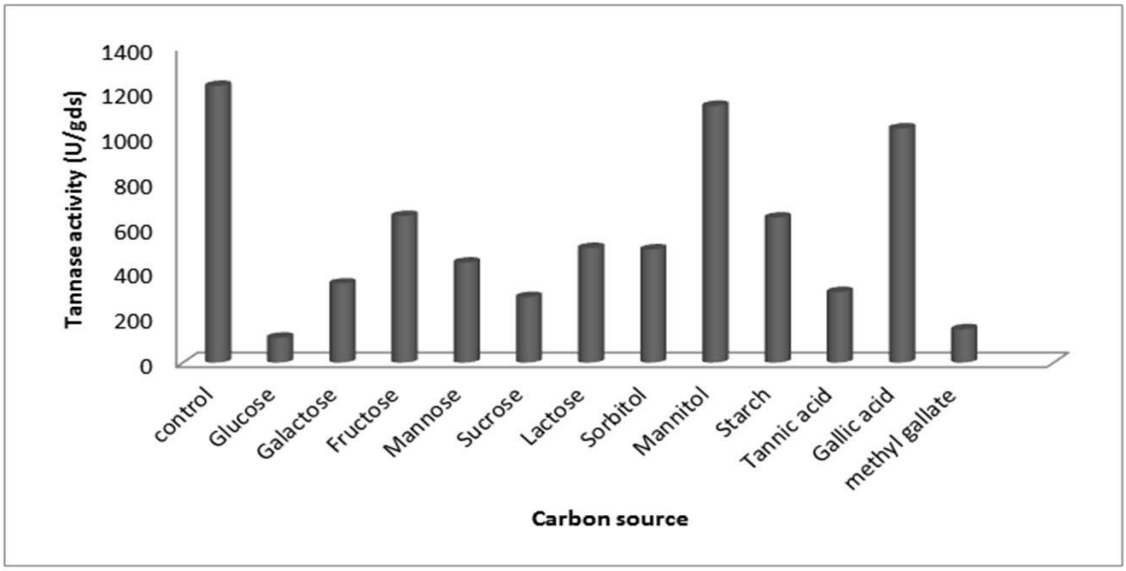

Fig. (2): Effect of different carbon sources supplemented to OC on production of *K. marxianus* NRRL Y-8281 tannase

It should be noted that the microorganism produces tannase enzyme to degrade tannic acid into gallic acid and glucose, the latter is utilized as carbon source for the microorganism growth [30]. In microorganisms, the term carbon catabolite repression refers to a phenomenon in which the presence of a rapidly metabolizable carbon source in the medium can repress expression of certain genes whose gene products often code for enzymes related to the utilization of alternative complex carbon sources [31]. This phenomenon

presents a good explanation to the inhibition of tannase production by the selected strain when glucose, sucrose, galactose, mannose, lactose, fructose and mannitol were added to the media (Fig.1).

Similar results were obtained stating *Enterobacter cloacae* tannase repression due to fructose, sucrose, glucose, galactose, mannose and lactose [9]. *Bacillus subtilis* PAB2 tannase was reported to be induced with glucose and mannose, while repressed with fructose and lactose when tamarind seed was used as sole carbon source for tannase production under SSF [32].

K. marxianus tannase was repressed by the addition of sorbitol and starch. The addition of an extra carbon source to the media changes the carbon/nitrogen ratio and creates an osmotic stress leading to cell growth inhibition, cell death or inhibition of enzyme synthesis [33]. Tannase inhibition by starch was also reported by Jana *et al.* [32].

Results in Fig.(2) revealed that addition of tannic acid resulted in repression of *K. marxianus* tannase production by 74.5%. High concentration of tannic acid (or tannins generally) binds irreversibly with microbial cell membrane proteins leading to impaired metabolism and inhibition of microbial growth and/or enzyme production, a phenomenon that is known as tannic acid toxicity [14, 34]. All previous reports ensured reduced tannase production at high concentrations of tannic acid [32, 35, 36]. Higher optimum concentration for tannic acid (2.5%) was reported when cashew apple bagasse was used for tannase production under SSF [27].

Also, results in Fig. (2) revealed that addition of gallic acid and methyl gallate resulted in reduced tannase production by 15.37 and 87.98%, respectively. Tannase activity is inhibited competitively by gallic acid [13]. Deposition of gallic acid on the cell surface results in reduction of tannase synthesis. These results agree with those reported by Rodrigues *et al.* [27].

All tested nitrogen sources showed inhibition of tannase production as shown in Fig. (3). This may mean that either OC is rich enough to supply nitrogen for the microbial cells to grow and produce enzymes [29], or the selected microorganism requires a low nitrogen level in order to produce tannase [37]. Maybe the addition of extra nitrogen source to the media resulted in imbalanced C/N ratio which affected the microbial growth and/or enzyme production [23].

Addition of organic nitrogen sources results in complexation of protein with tannins in the media, leading to minimal microbial growth and lower tannase production [14]. This presents a good caption for tannase repression by peptone, yeast extract and malt extract (Fig.3). Tannase induction by yeast extract, malt extract and peptone supplementation was previously reported [32]. On the other hand, tannase reduction by yeast extract and peptone was stated by Rodrigues *et al.* [27] and Lal and Gardner [38].

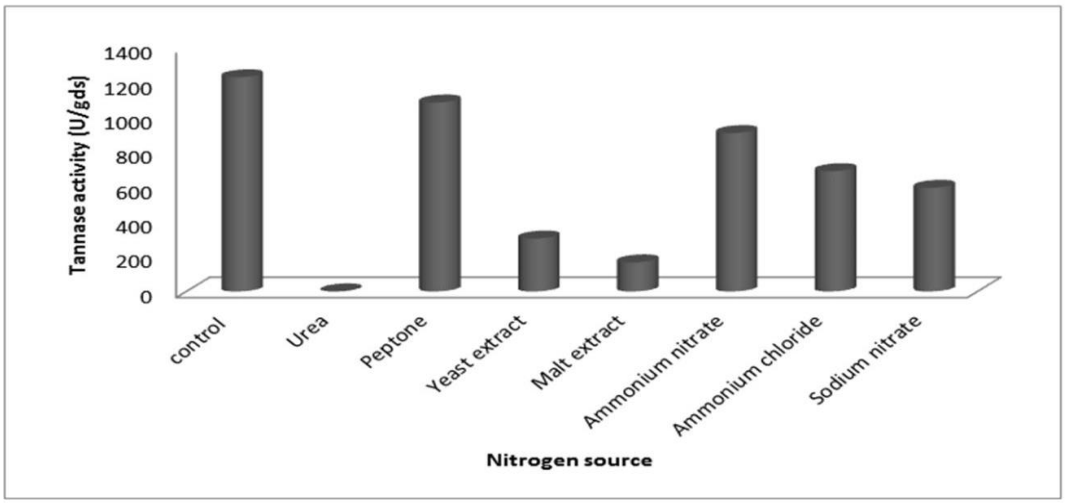

Fig. (3): Effect of different nitrogen sources on production of *K. marxianus* NRRL Y-8281 tannase

Also, the addition of inorganic nitrogen sources (sodium nitrate, ammonium nitrate and ammonium chloride) resulted in reduced tannase production as shown in Fig. (3). Ammonium nitrate and sodium nitrate mediated induction of tannase was reported by Jana *et al.* [32], while tannase repression by both nitrogen sources was reported by [23].

The inhibitory effect of ammonium chloride on tannase production was also reported by Sabu *et al.* [23] and Sivashanmugam and Jayaraman [39]. However tannase induction as a result of ammonium chloride supplementation was reported by Beniwal *et al.* [9].

Being a protein, tannase enzyme activity is strongly affected by the pH. This is because the ionic character of the amino and carboxylic acid groups of the amino acids responsible for the active site formation is changed under different pH values due to protonation and deprotonation processes [29].

It should be noted that both substrate (tannic acid) and product (gallic acid) of tannase create an acidic environment thus, fermentation at inappropriate pH was proved to be detrimental [14]. Tannases have been reported to be acidic proteins, with an optimum pH around 5.5 [29].

Each microorganism expresses optimal tannase productivity at a certain pH [40]. The pH of raw OC was estimated to be 6.0 which coincides with that reported by Brlek *et al.* [41] and Lafka *et al.* [42] and this pH was found to be optimum for maximum tannase production by *K. marxianus* (Fig. 4). This pH is within the pH growth range of the selected strain since *K. marxianus* can grow at pH range of 2.5 - 8.0 [43]. Similar results were reported by Jana *et al.* [32]; Sivashanmugam and Jayaraman [39] and Raghuwanshi *et al.* [44].

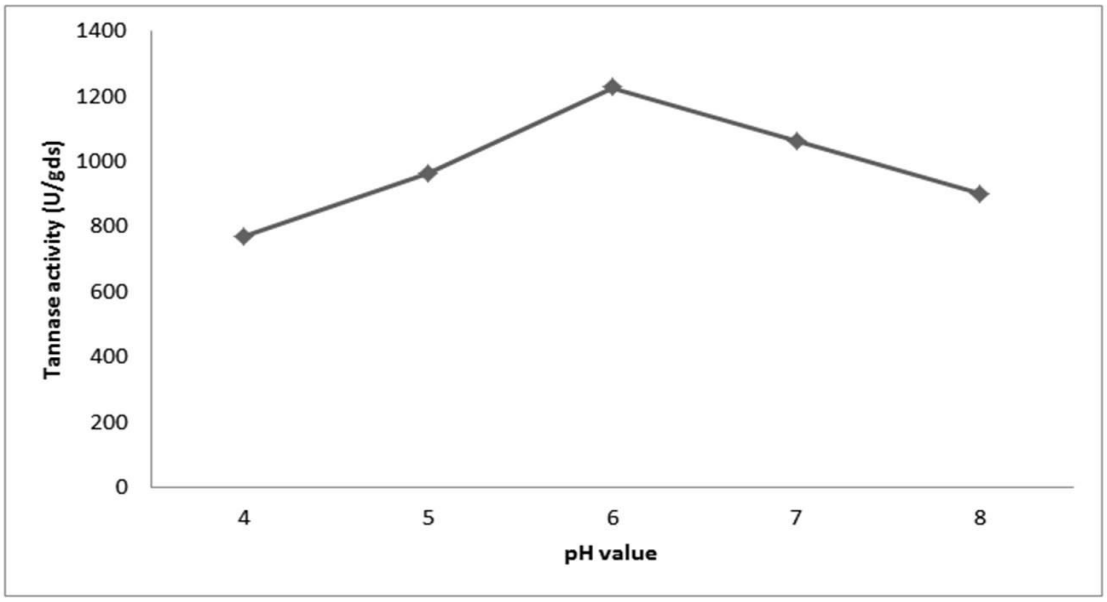

Fig. (4): Effect of initial pH on production of *K. marxianus* NRRL Y-8281 tannase

On proceeding toward optimum temperature for tannase production, the enzyme level increases gradually due to the increased reaction rate resulted from increased kinetic energy of reacting molecules. At temperature higher than the optimum one, thermal denaturation of metabolic pathway occurs resulting in poorer production of metabolites [32].

Results shown in Fig. (5) showed that 45°C was found to be the optimum temperature for tannase production by *K. marxianus* giving 1714.74 U/gds. Below and above this temperature the enzyme level was decreased. It should be noted that *K. marxianus* can grow at different temperatures ranging from 1 to 47°C, above that temperature the yeast cells fail to grow. The ability to grow at high temperature is one of the hallmarks of *K. marxianus* [43].

ISSN: 0975-8585

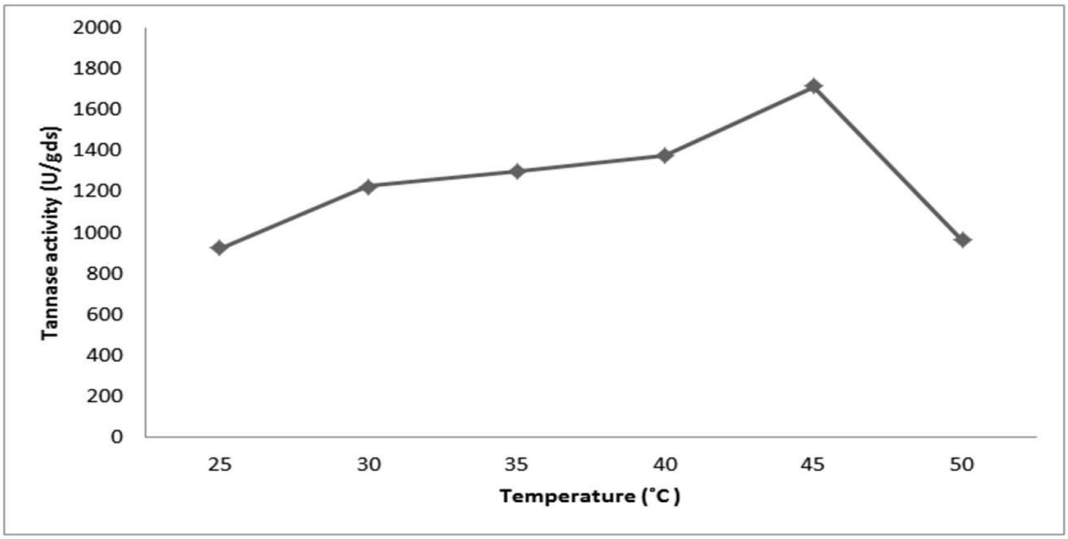

Fig. (5): Effect of incubation temperature on production of *K. marxianus* NRRL Y-8281 tannase

Implementation of fermentation at higher temperatures not only reduces significantly the cooling cost in large scale production, but also reduces the contamination risk [45]. Thus the thermo-tolerant yeast *K. marxianus* can be considered as a promising biotechnological tool for tannase production.

The optimum inoculum size of the *K. marxianus* that gives maximum tannase yield as shown in Fig. (6) was found to be 20% which is similar to the result of Sabu *et al.* [46] who used *Lactobacillus* sp. ASR-S1 for SSF of coffee husk. Beniwal *et al.,* [9] reported 1% of *Enterobacter Cloacae* MTCC 9125 to be the optimum inoculum size while Raghuwanshi *et al.* [44] stated an optimum inoculum level of 2%.

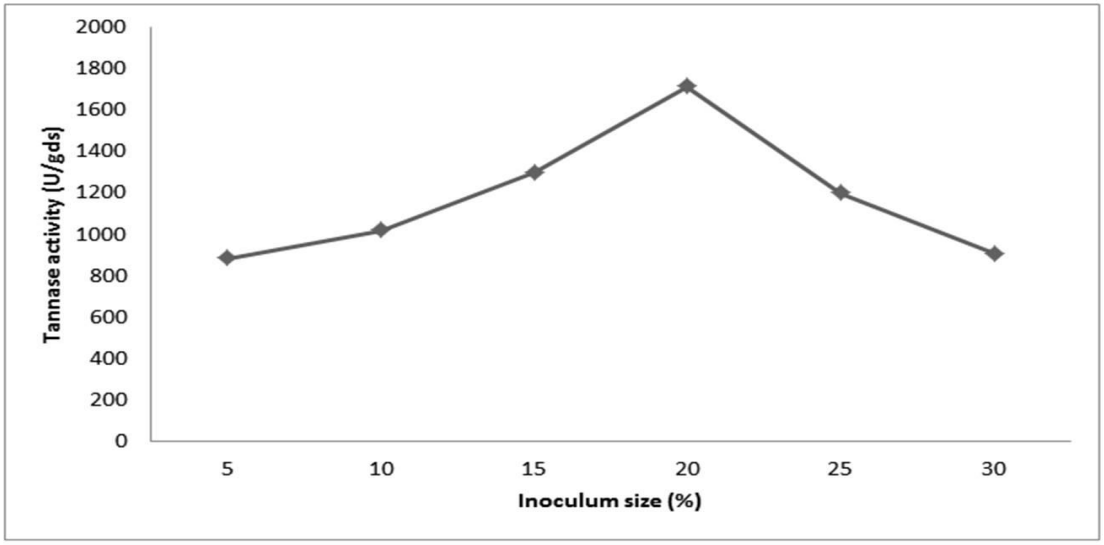

Fig. (6): Effect of inoculum size on production of *K. marxianus* NRRL Y-8281 tannase

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The definition of SSF normally involves the microbial growth on solid support in the lack or near absence of free water. Nevertheless, the substrate used as solid support must contain sufficient moisture which is available in the absorbed form within the solid support [47]. Increasing or decreasing the moisture content than the optimum level may result in reduced enzyme production due to osmotic imbalance inside the microbial cells [28].

The optimum moisture content for *K. marxianus* tannase production was found to be 35% giving maximum enzyme yield of 1714.7 U/gds. Lower and higher moisture contents resulted in inhibition of tannase production by a ratio of 0.9-74% as shown in Fig. (7). A moisture level of 44% was optimum when palm kernel cake and tamarind seed powder were used as solid support for *Lactobacillus* sp. fermentation [46]. While when *A. niger* was used for fermentation, the optimum moisture level changed to 53.5% for palm kernel cake and 65.8% for tamarind seed powder [23].

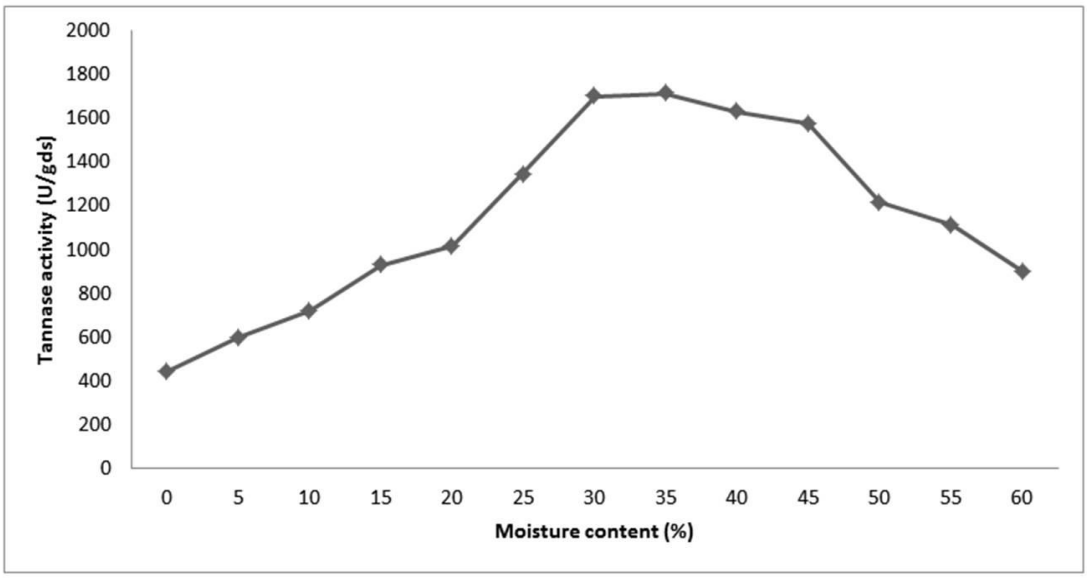

Fig. (7): Effect of moisture content on production of *K. marxianus* NRRL Y-8281tannase

However higher moisture level such as 80% was reported by [28] using *Terminalia chebula* powder and powdered pod cover of *Caesalpinia digyna* as solid support for a co-culture of the filamentous fungi, *Rhizopus oryzae* and *A. foetidus*.

It should be noted that lower moisture content is more preferred industrially because the lower the moisture level, the smaller the fermenter is required. In addition the product produced is more concentrated leading to a reduction of downstream processing and effluent treatment. Also lower free water results in lower sterilization costs [48].

Cost effective tannase production remains a main obstacle for biotechnologists, due to its high processing cost mainly due to raw substrate, tannic acid [14]. So the production of tannase by fermentation without addition of external additives reduces the cost of its production representing more economical trend for industrial tannase production.

To the best of our knowledge, this is the highest tannase yield (1714.7 U/gds) obtained among different fermentation techniques. Also, this is the first report concerning tannase production using either the yeast *K. marxianus* or the agro-industrial waste OC.

REFERENCES

[1] Dermeche S, Nadour M, Larroche C, Moulti-Mati F, Michaud P. Process Biochem 2013; 48: 1532-52.
[2] Alu'datt MH, Alli I, Ereifej K, Alhamad M, Al-Tawaha AR, Rababah T. Food Chem 2010; 123: 117-22.
[3] Ravindran R, Jaiswal AK. Trends biotechnol 2016; 34: 58-69.
[4] Christoforou E, Fokaides PA. Waste Manage 2016; 49: 346-63.
[5] Govindarajan R, Revathi S, Rameshkumar N, Krishnan M, Kayalvizhi N. Biocatal Agric Biotechnol 2016; 6: 168-75.
[6] Lata S, Rani KP. J Pharm Biol Chem Sci 2016; 7: 1471-85.
[7] Belur P, Mugeraya G. Res J Microbiol 2011; 6: 25-40.

[8] Venu Gopal KSCC, Anu Appaiah KA. Curr Biochem Eng 2016; 3: 82-88.
[9] Beniwal V, Chhokar V, Singh N, Sharma J. J Am Sci 2010; 6: 389-397.
[10] Beniwal V, Kumar A, Sharma J, Chhokar V. Recent pat biotechnol 2013; 7: 228-233.
[11] Lata S, Rani P. Adv App Sci Res 2015; 6: 110-116.
[12] da Costa AM, de Souza CGM, Bracht A, Kimiko M, Kadowaki ACdSd, Souza RFO, et al. Afr J Biochem Res 2013; 7: 197-202.
[13] Chávez-González M, Rodríguez-Durán LV, Balagurusamy N, Prado-Barragán A, Rodríguez R, Contreras JC, et al. Food Bioprocess Technol 2012; 5: 445-459.
[14] Jana A, Halder SK, Banerjee A, Paul T, Pati BR, Mondal KC, et al. Bioresour technol 2014; 157: 327-340.
[15] Rodríguez-Durán LV, Valdivia-Urdiales B, Contreras-Esquivel JC, Rodríguez-Herrera R, Aguilar CN. Enzyme res 2011; 2011: 1-20.
[16] Wickerham LJ. Taxonomy of yeasts: US Dept. of Agriculture; 1951.
[17] Jodon M, Royse D. Research report 1979.
[18] Iibuchi S, Minoda Y, Yamada K. Agric Biol Chem 1967; 31: 513-518.
[19] Bhat TK, Singh B, Sharma OP. Biodegradation 1998; 9: 343-357.
[20] Cruz R, de Lima JS, Fonseca JC, dos Santos Fernandes MJ, Lima DMM, Duda GP, et al. Adv Microbiol 2013; 3: 1-52.
[21] Lima JSd, Cruz R, Fonseca JC, Medeiros EVd, Maciel MdHC, Moreira KA, et al. Sci World J 2014; 2014: 1-9.
[22] Paranthaman R, Vidyalakshmi R, Murugesh S, Singaravadivel K. Afr J Microbiol Res 2010; 4: 1440-1445.
[23] Sabu A, Pandey A, Daud MJ, Szakacs G. Bioresour Technol 2005; 96: 1223-1228.
[24] Selwal MK, Yadav A, Selwal KK, Aggarwal N, Gupta R, Gautam S. World J Microbiol Biotechnol 2010; 26: 599-605.
[25] Lokeswari N. International Journal of Pharmacy 2012; 2: 375-379.
[26] Paranthaman R, Vidyalakshmi R, Murugesh S, Singaravadivel K. Global J Biotechnol Biochem 2008; 3: 105-110.
[27] Rodrigues TH, Dantas MAA, Pinto GA, Gonçalves LR. Appl Biochem Biotecnol 2007; 675-688.
[28] Banerjee R, Mukherjee G, Patra KC. Bioresour Technol 2005; 96: 949-953.
[29] Kumar R, Sharma J, Singh R. Microbiol Res 2007; 162: 384-390.
[30] Bradoo S, Gupta R, Saxena R. Process Biochem 1997; 32: 135-139.
[31] Jankovic I, Bruckner R. J mol microbiol biotechnol 2002; 4: 309-314.
[32] Jana A, Maity C, Halder SK, Mondal KC, Pati BR, Mohapatra PKD. Biocatal Agric Biotechnol 2013; 2: 363-371.
[33] Rodríguez H, de las Rivas B, Gómez-Cordovés C, Muñoz R. Int j food microbiol 2008; 121: 92-98.
[34] Paranthaman R, Vidyalakshmi R, Murugesh S, Singaravadivel K. Glob J Biotechnol Biochem 2009; 4: 29-36.
[35] Aboubakr HA, El-Sahn MA, El-Banna AA. Brazilian Journal of Microbiology 2013; 44: 559-567.
[36] Riul AJ, Goncalves HB, Jorge JA, Guimarães LHS. J Mol Catal 2013; 85: 126-133.
[37] Patel R, Dodia M, Singh SP. Process Biochem 2005; 40: 3569-3575.
[38] Lal D, Gardner JJ. Eur J Exp Biol 2012; 2: 1430-1438.
[39] Sivashanmugam K, Jayaraman G. Afr J Biotechnol 2011; 10: 1364-1374.
[40] Kaur H, Dutt D, Tyagi C. BioResources 2011; 6: 1376-1391.
[41] Brlek T, Voća N, Krička T, Lević J, Vukmirović Đ, Čolović R. Agric Conspec Sci 2012; 77: 31-35.
[42] Lafka T-I, Lazou AE, Sinanoglou VJ, Lazos ES. Food Chem 2011; 125: 92-98.
[43] Kurtzman CP, Fell JW, Boekhout T, Robert V. The yeasts, a taxonomic study, 5th edn Elsevier, Amsterdam 2011: 87-110.
[44] Raghuwanshi S, Dutt K, Gupta P, Misra S, Saxena RK. J biosci bioeng 2011; 111: 635-640.
[45] Yang C, Hu S, Zhu S, Wang D, Gao X, Hong J. World J Microbiol Biotechnol 2015; 31: 1641-1646.
[46] Sabu A, Augur C, Swati C, Pandey A. Process Biochem 2006; 41: 575-580.
[47] Pandey A. Process Biochem 1991; 26: 355-361.
[48] Robinson T, Nigam P. Biochem Eng J 2003; 13: 197-203.