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DETERMINING THE BEST SPECIES FOR EXTRACTING AMYLASE ENZYME, THE DEVELOPMENT OF THE ENZYME DURING GERMINATION PERIODS, AND DIAGNOSING THE BIOACTIVE COMPOUNDS USING INFRARED SPECTROPHOTOMETRY

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ABSTRACT

A study was conducted to extract amylase enzyme from four species of cereals before and after germination, which were barley, wheat, local millet, and sorghum. The results of estimating the specific and enzymatic activity of all the studied species indicated that the best species for extracting the enzyme is sorghum, although barley malt is the most common in extracting this enzyme, as well as the two types of wheat and millet, which also gave effective values. The study recommended the use of sorghum in extracting the amylase enzyme as a forage crop with high economic feasibility. The results showed the highest qualitative effectiveness before and after germination (2255.56 and 6986.92 unit mg^{-1} protein), respectively, compared with the other of the studied species. Four cultivars of sorghum (Kaffir, Engath, Rabeh, and Geza) were selected to monitor the development of amylase activity during different germination periods (24, 48, 72, 96, and 120 hours) and by two methods, the effectiveness was estimated, namely Wilson and DNSA. The results showed that the highest enzymatic and specific activity of Kaffir cultivar in germination times 24, 72, and 96 hours by DNCs method (253.2, 226.80, and 276.84 unit mg^{-1} protein), respectively. The results of the infrared spectrum analysis of the sorghum for the two Kaffir and Geza cultivars during the germination times 24, 72, and 96 hours showed the presence of the bioactive groups in the chemical compounds. It was noted that these groups do not change from one compound to another and are important in identifying the molecule to their role in giving the physicochemical properties.

Keywords: α-amylase; bioactive compounds; enzyme activity; potency assessment; sorghum.

INTRODUCTION

Enzymes have witnessed a great development in the food industry as nutritional and industrial supplements because of their wide spread and can be used in a range of nutritional processes. Enzymes were produced from various sources, including plants, animals and microorganisms, as they are the most studied enzymes [1]. The enzyme was extracted from microbial, bacterial and fungal sources, in addition to plant and animal sources. In general, enzymes have been widely used in the fields of food manufacturing, especially amylase enzymes, because of their importance in improving the quality of baked goods, and it is considered safe and healthy and does not harm human health [2]. Alpha-amylase enzyme is one of the oldest enzymes used by humans in various fields, the most important of which was the field of food and pharmaceutical industries. It was used in the analysis of starch to produce glucose syrup, as it was used in the production of alcohol and spirits, and its use in improving bread and producing baby food. It is also one of the commercially important enzymes for its ability to decompose starchy materials into simple sugars such as dextrin, maltose and glucose, which are important sugars in the food industry [1]. Amylase enzymes are classified in the group of hydrolysis enzymes and are called by this name because they work on the breakdown of amylose. The sources of alpha-amylase enzyme production varied, and the enzyme was first extracted from barley malt, then several attempts were made to produce it from microorganisms. Research has continued up to the present time, as the enzyme is produced in large quantities from different sources, and in the world there are 3000 types of enzymes. The use of them in different fields is only 1% of these types. These enzymes are produced in quantities estimated at thousands of tons annually, and this reflects the growing interest in enzymes because they have an important role in most different areas of life [3]. Because amylase enzymes increase significantly in sprouted grains, many researchers have produced α-amylase enzyme from different sprouted grains of barley, wheat, sorghum and millet, studying its properties and using it in various food industries, such as using malt with high amylase activity as a bread improver, and in the production of infant meals as an aid in the analysis of starchy materials [4]. The aim of this study is to extract the amylase enzyme from the sources of forage grain crops because it is of high economic feasibility, and to monitor the activity of the enzyme during the germination process and the development of some effective chemical groups during the germination periods.

MATERIALS AND METHODS

Germination Process

Seed germination process was conducted in Petri dishes according to the method described in Abdelmoneim et al. [5]. A quantity of sorghum grains was taken and soaked in distilled water for 24 hours at a temperature of 24°C. Then germination was carried out through the use of petri dishes, as a filter paper was placed at the base of each petri dish and 30 grains were placed on it. The dishes were incubated at 24°C for 4 days, taking into account the moistening of the grains with a little water during the germination stages, and the activity of the α-amylase enzyme was monitored during those stages.

Determination of Amylase Activity

The activity of amylase was evaluated in two ways:

1. The first method using the method Wilson and Ingledew [6] on the basis of the enzymatic unit: it is the amount of enzyme that dissolves 0.1 mg of the starch subject during a period of ten minutes at a temperature of 40 ° C when the concentration of the subject substance is 4 (mg ml^{-1}).
2. As for the second method, the activity of the enzyme was estimated by the method of the reagent di nitro cellulose (DNCs) according to the method described in Kikani and Singh [7].

Protein Content Determination

The protein content of cereals was estimated during different stages of the study depending on the method of Lowry et al. [8].

Infrared Spectroscopy Diagnostics

The sample was prepared by mixing the samples under study with potassium bromide (Kerr), and it shaped in the form of discs and mixed well, and placed in its designated place in the FT-IR infrared device model Shimadzu Affinity-1 FTIR Spectrophotometer of the College of Education for Pure Sciences, College of Education in University of Basrah.

Experimental Design and Statistical Analysis

Experiments are designed according to Randomized Complete Design. The data were analyzed using the ready-made statistical program SPSS Version 24. The mean of the treatments were compared according to the Revised Least Significant Difference Test at a probability level of 5%.

RESULTS AND DISCUSSION

Determining the Best Type to Extract the Amylase Enzyme from the Grains

The results obtained in Fig. 1 indicated the qualitative activity of the species of cereals under study before and after germination of barley, wheat, local millet and sorghum. It was noticed through the statistical analysis that there were significant differences at the 5% probability level. The results in Fig. 2 showed that there was a specific activity for all the species under study, as it gave sorghum the highest specific activity before germination, which amounted to 2255.39 unit mg^{-1} proteins, and the specific activity after germination amounted to 6986.62 unit mg^{-1} protein. Followed by barley species before germination, the specific activity reached 723.85 units mg^{-1} protein, while after germination; its specific activity reached 1024.61 units mg^{-1} protein. Then it was followed by non-germinated wheat grains with a specific activity of 586.51 unit mg^{-1} protein and after germination with specific activity of 1047.64 unit mg^{-1} proteins. The last of which was the local non-germinated millet with a specific activity of 211.38 unit mg^{-1} protein and after germination its specific activity reached 967.91 units mg^{-1} proteins. The results from the same table show the significant superiority of sorghum grains over the other species. Although the species of barley was considered the main source for extracting the amylase enzyme, it was excluded due to its frequent use in extracting this enzyme. According to these results, the sorghum species was chosen as it is an available vegetable feed source from which the amylase enzyme can be extracted as it decomposes the starch into smaller units of glucose, maltose and dextrin. Distilled water at laboratory temperature was used to extract unwanted polysaccharides and phenolic compounds. These results are in agreement with the findings of Que [9].

Monitoring the Activity of Amylase during the Germination Period

After that, sorghum species was determined to extract the enzyme, where four cultivars of sorghum were selected: Kaffir, Engath, Rabeh, and Geza. Four germination times were determined, 24, 48, 72 and 96 hours, and two methods were used for the purpose of estimating the enzymatic activity using DNCs and Wilson methods [6,7].

The results in Fig. 3 showed the enzymatic and specific activity values of the cultivars under study, with significant differences at the 0.05% probability level for all germination times. The effectiveness values of the DNCs method were superior to that of the Wilson method for all germination times. The highest enzymatic activity value of Kaffir cultivar was in germination time of 96 hours, which amounted to 276.84 unit ml^{-1}, and the lowest was in the non-germinated in 24 hours, which amounted to 211.001 unit ml^{-1}. Through the values of the different enzymatic activity in the times of germination, it was found that the values of the two cultivars Engath and Rabeh are slightly close for all times, as the values of the enzymatic activity at germination times of 24, 48, 72 and 96 hours for Engath cultivar are as follows: 150.101, 181.124, 240.916 and 225.194 unit ml^{-1}, respectively, while the values of Rabeh cultivar reached 132.101, 181.124, 180.12 and 253.416 unit ml^{-1}, respectively in the same germination times. The lowest values for the enzyme activity of Geza cultivar, according to the germination times 24, 48, 72 and 96 hours, were 111.101, 133.555, 175.012 and 175.242 unit ml^{-1}, respectively.

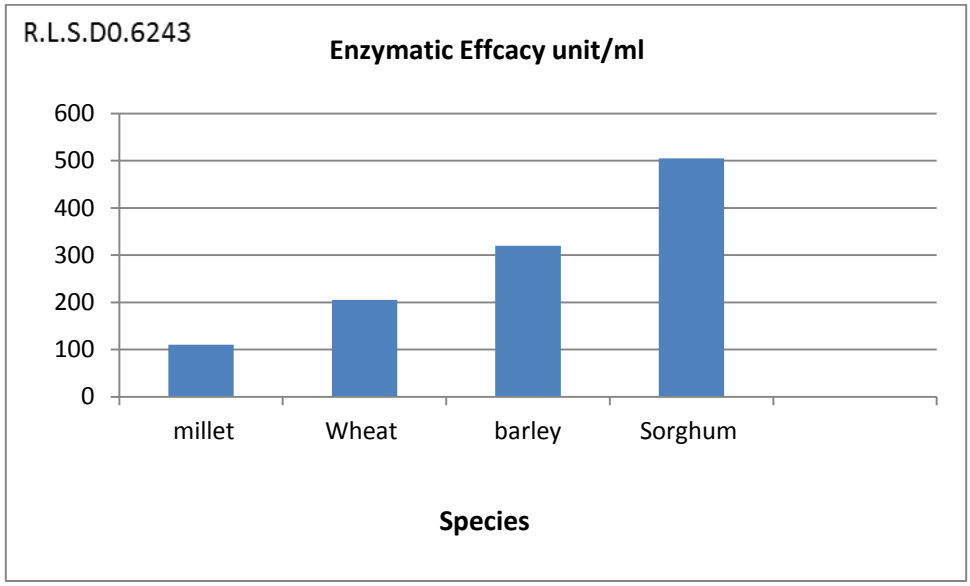


Fig. 1. Determining the best species for extracting amylase

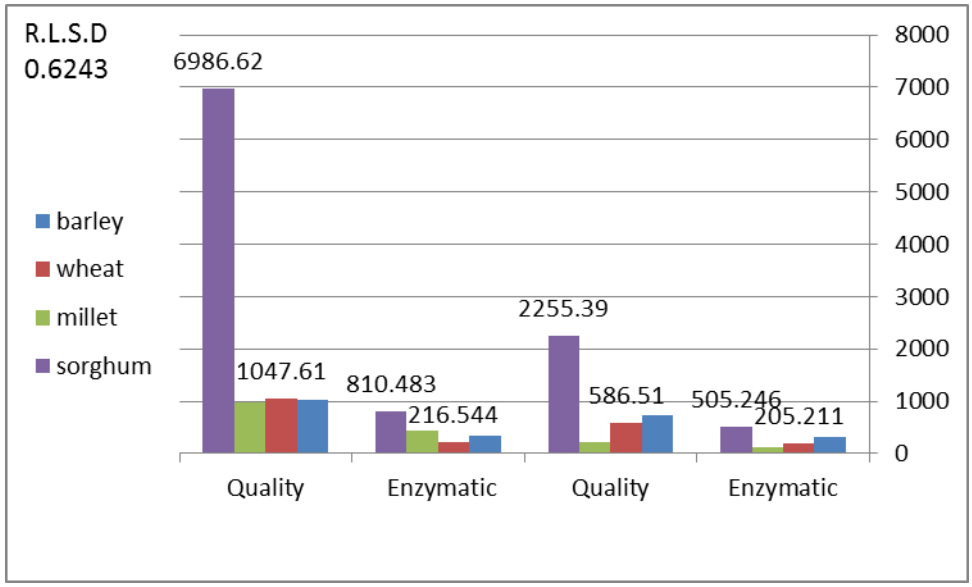
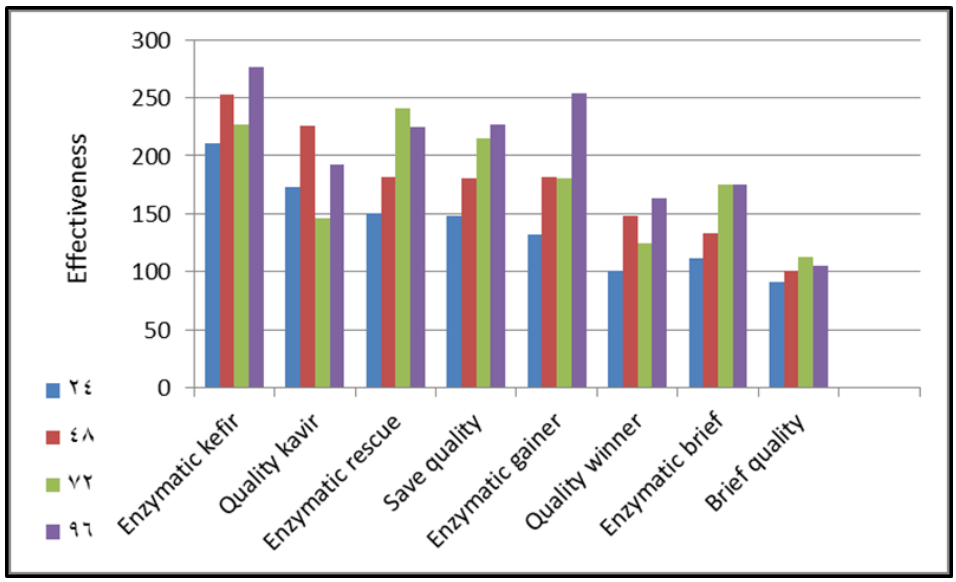


Fig. 2. The specific and enzymatic activity values to choose the best cultivar for extraction of the amylase enzyme

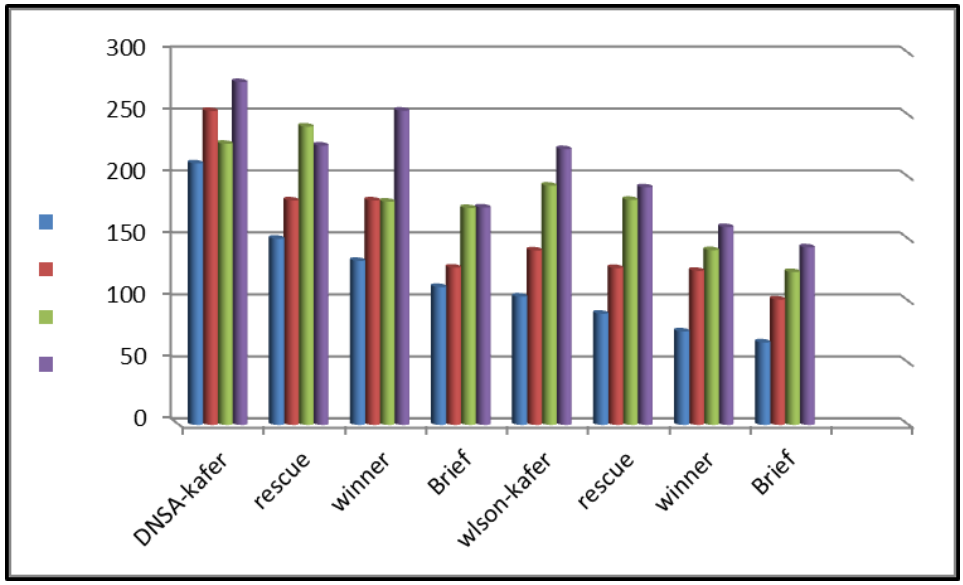
When estimating the enzymatic and specific activity by Wilson method, the enzymatic activity values of the cultivars under study were in an ascending manner until germination time of 96 hours. Then the effectiveness began to decrease gradually as the germination period continues, in order to consume all the starches in the germinated grains. The results in Table 1 and the statistical analysis at the 0.01 probability level show that the best effectiveness values were for Kaffir cultivar for all germination times by the DNCs method, and then followed by an Engath,

Rabeh and Geza, respectively. All the cultivars under study gave different enzymatic activity due to the different cultivar, cultural conditions and genetic characteristics of the plants under study. It was found that the values of enzymatic activity differ from one cultivar to another, according to the germination times and the method of estimation. As the process of soaking and germination has a different effect on the values of enzymatic activity. These results agreed with the findings of many researchers [7,10,11].



RLSD (96)0.301 .RLSD(72)0.123 RLSD (48)3.874 RLSD (24)0.2490

Fig. 3. The enzymatic activity and specificity values by the DNCs estimation method



RLSD (96)0.301. RLSD (72)0.123 RLSD(48)3.874 RLSD (24)0.2490

Fig. 4. Shows the enzymatic activity and specificity values by the DNSD estimation method

FTIR Diagnostics

The sorghum of Kaffir and Khazra cultivars was diagnosed with germination times 24, 72 and 96 hours by infrared spectrophotometer to diagnose the effective groups present in sorghum cultivars as shown in Figs. (5-11) and Table 1 which shows the infrared rays for the germination times, as they gave Wide bands at the frequency (3396.64-3522.02-3412.08 cm^{-1}) are due to the presence of water (OH str.vibranu) and a COOH carboxylic group, which is the region where the absorption of the active groups occurs. While the band at frequency 2927.94-2858.51 cm^{-1} that returns the amplitude oscillation of aliphatic C-H of CH, CH3 and S=O groups. While the band at 1797.66-1745.58 cm^{-1} which is due to the amplitude oscillation of the ketone and aldehyde group C and the appearance of the band belongs to the alkane group C = C at the frequency of 1651.07 cm^{-1}. While the frequency that appeared at 1539.20-1510.26 cm^{-1}, which are the regions of the pair bonds that belong to C=N C=O and C=C.

It was noted that the frequency is at 1423.47-1024.20 cm^{-1}, which is located within the fourth region of the frequency in which the expansion of bonds, bending and absorption of colloidal bonds between carbon atoms and other non-hydrogen atoms C-CL, C-C and C=O. While the bands that appeared at the frequency 933.55-520.78 cm^{-1}, which are low-frequency areas, showed bands of fats, carbohydrates, starch, protein and phenol. It showed an increase in firmness when germination time was increased 72 and 96 hours with an increase in the vibrational curvature of the starch.

Table 1. Shows the effective groups for the infrared spectrum analysis for the grains under study

The amplitude vibration of the effective groups The waveforms are -1 cm									Chemical groups
C-O;C-C 1151,900	**C-O-H,C-C-H,C-O-H 1400-1150**	**Amide IIN-H,C-N1538**	**Amide IC=O 1652**	**Carboxyl COOH 1709**	**Ester C=O 1745**	**Methl-CH_3 2854**	**Methylene-CH_2 2925**	**OH,N-H 3316.5**	**Totals**
1157.29 1080.14 1043.49 1024.20 933.55	1342.46 1246.02 1157.29	1539.20	1651.07		1745.58	2858.51	2927.94	3522.02 3412.08 3396.64	Standard sorghum grain
1157.29 1080.14 1024.20 931.62	1425.40 1244.09	1516.05	1658.78	-	1797.66	2856.58 2517.10	2926.01	3408.22 3373.50 3010.88	Kefir is not germinated
1157.29 1082.07 1020.34 933.55	1419.61 1377.71 1242.16	1537.27 1516.05	1649.41		1743.65	2854.65	2926.01	3367.71 3344.57	kefir germinated 72
1155.36 1080.14 1022.27 931.62	1425.40 1242.16	-	1656.85	-	-	2858.51	2927.94	3414.00 3392.79	Kefir germinated 96
1159.22 1082.07 1018.41 931.62	1454.33 1423.47 1375.25 1244.09	1539.20 1517.98	1653.00	-	-	2827.58	2927.94	3398.57 3371.57	brief not grown 24
1156.29 1080.14 1024.20 931.62	1423.47 1244.09	-	1656.85	-	1743.65	2856.56	2926.01	3406.50 3346.50	Brief germination 72
1157.29 1080.14 1020.34 929.69	1419.61 1375.25 1340.53 1242.16	1541.12 1519.91	1651.07		1745.58	2854.65	2926.01	3392.79 3363.86 3342.64 3010.88	Brief germination 96

The above results show that the active groups of the grains under study are aliphatic chains belonging to fat, protein, starch and phenolic acid. These results agree with the findings of many researchers [12,13].

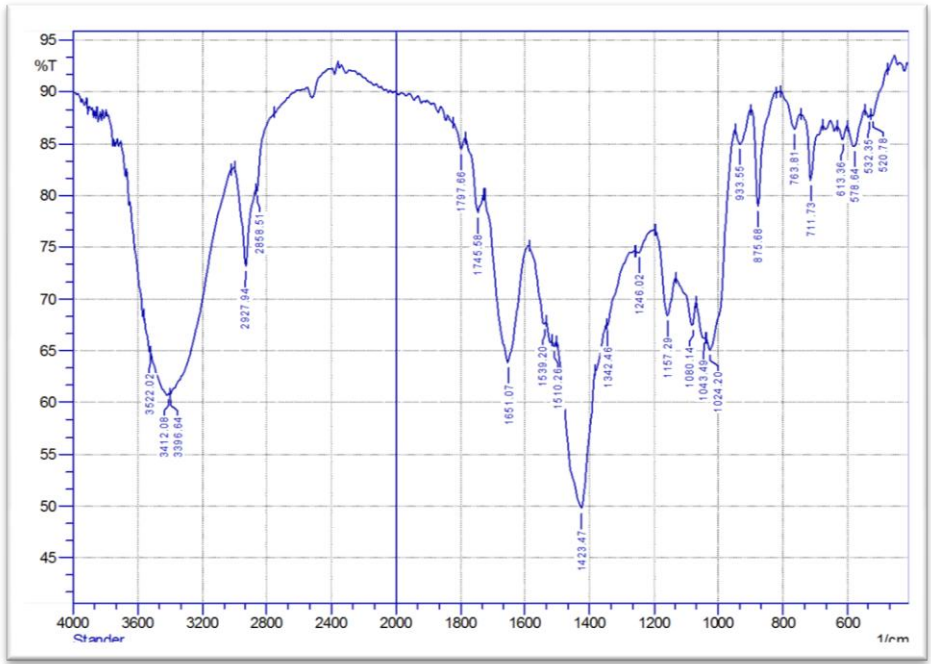


Fig. 5. Standard white maize variety from the Agricultural Research Department, Baghdad

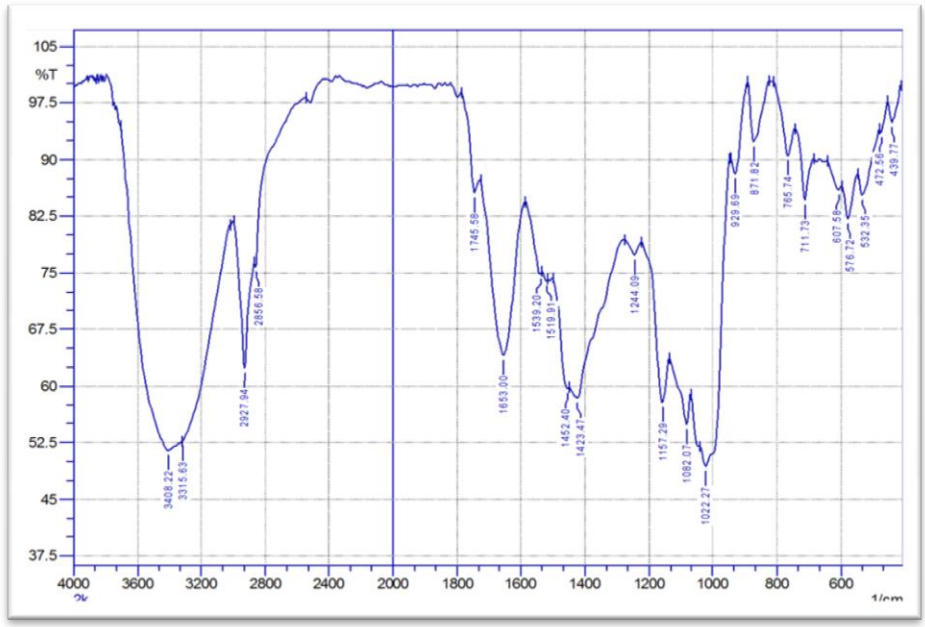


Fig. 6. Kaffir variety not sprouted

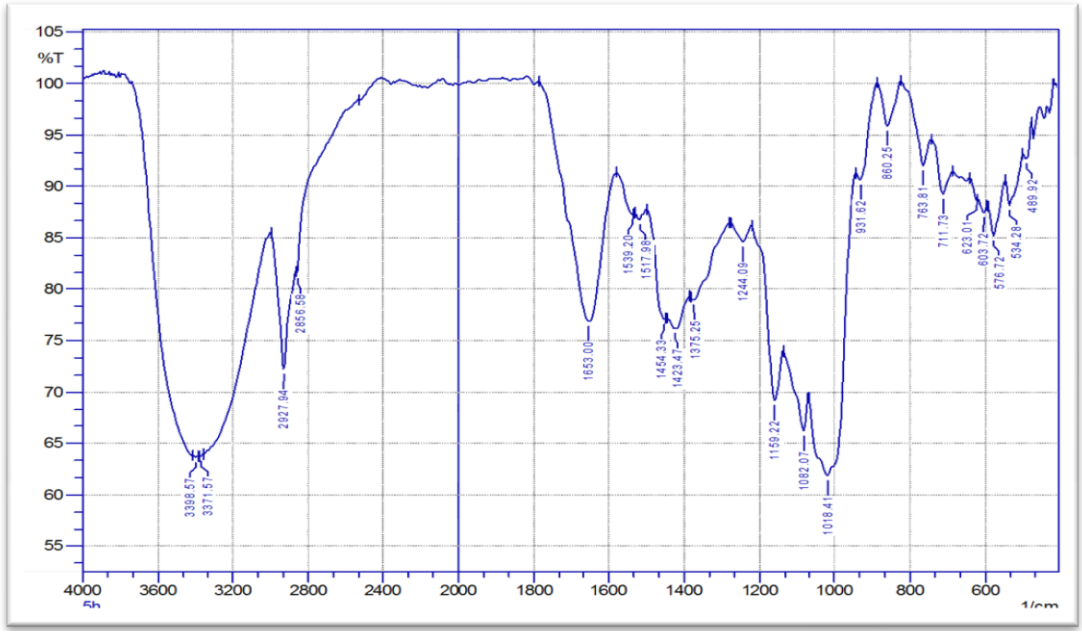


Fig. 7. Kaffir germinated 72 hours

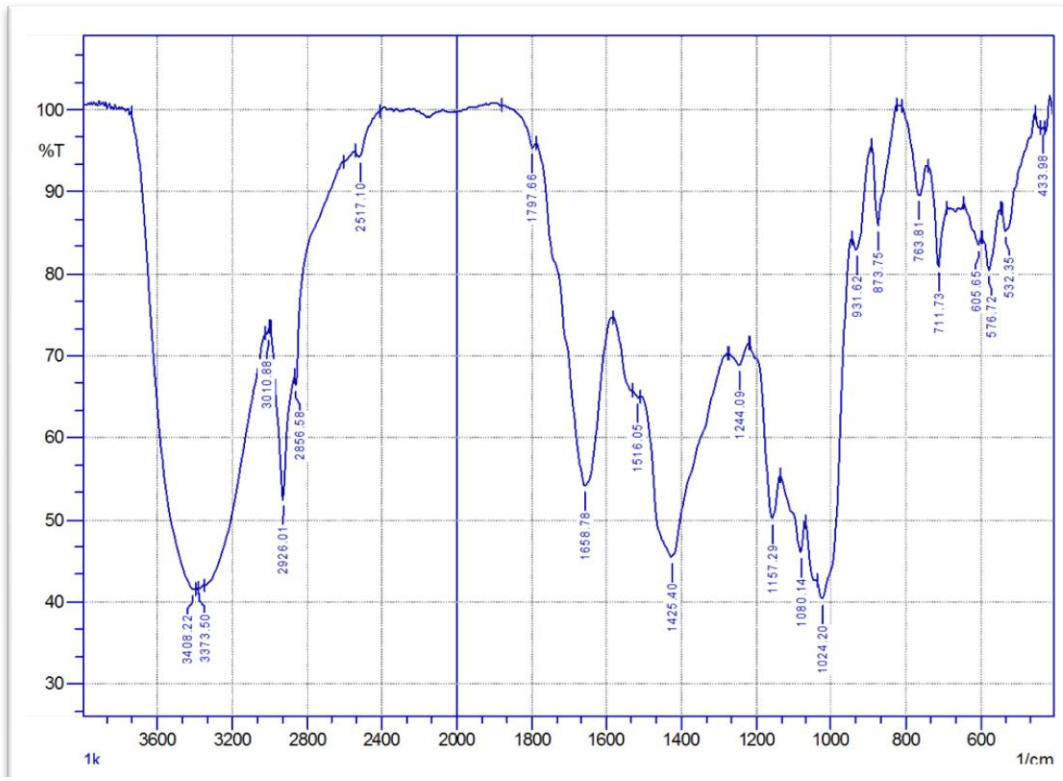


Fig. 8. Kaffir germinated 96 hours

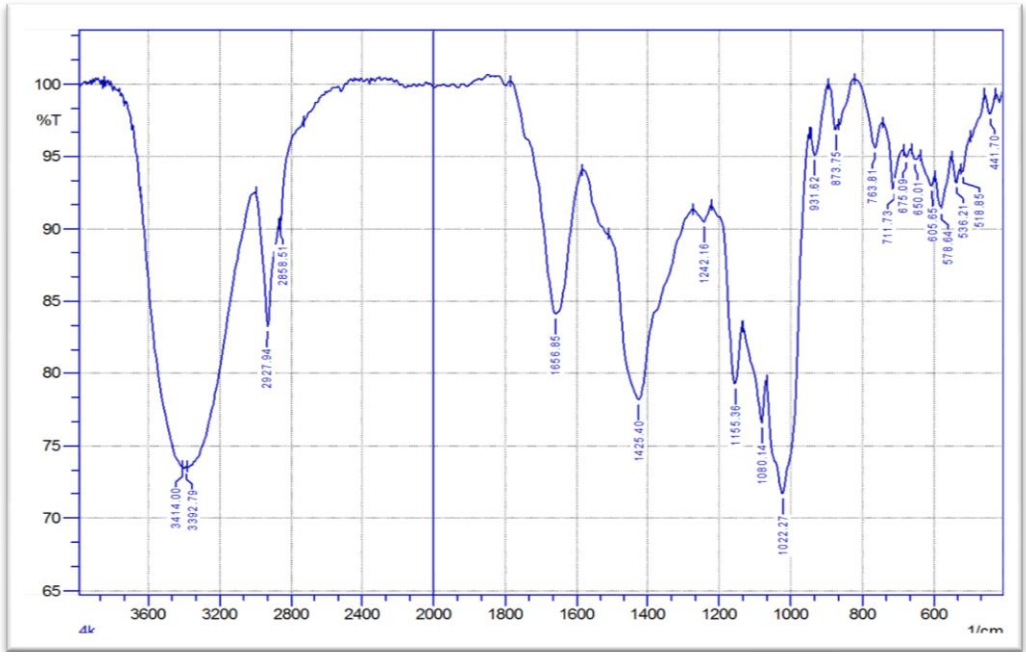


Fig. 9. Brief variety is not grown

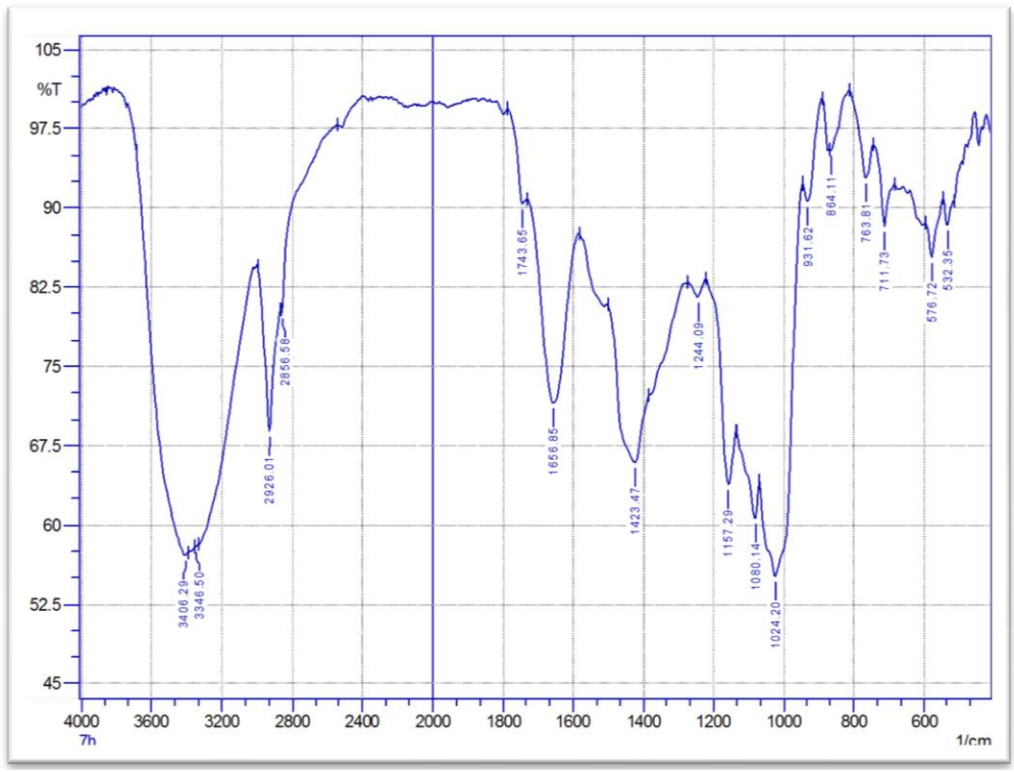


Fig. 10. Brief germinated 72 hours

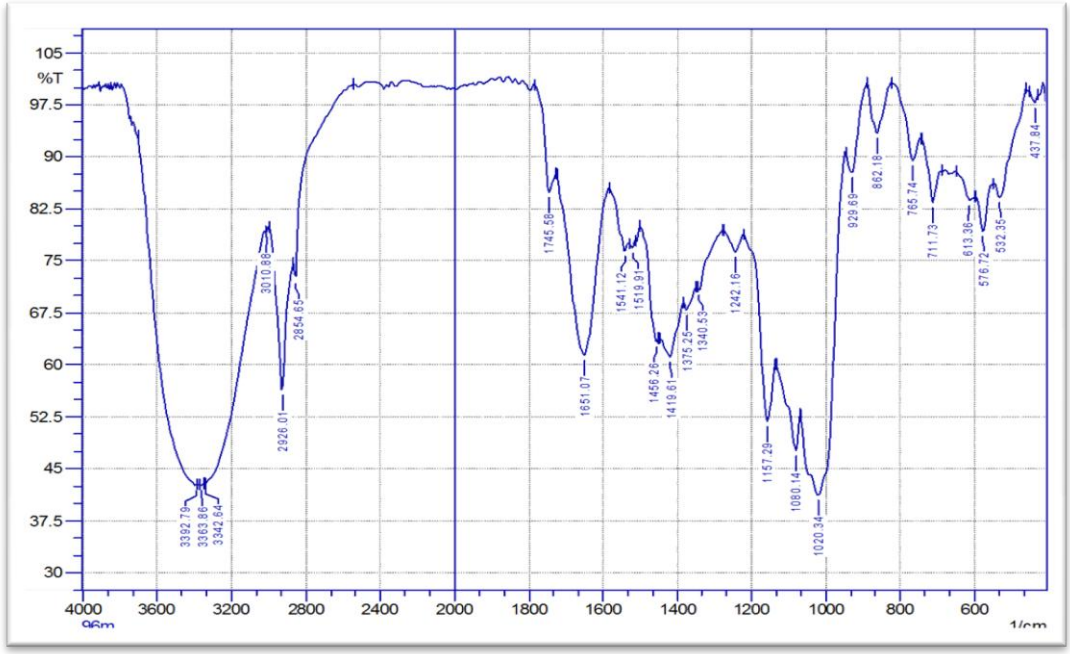


Fig. 11. Brief germinated 96 hours

CONCLUSION

The specific and enzymatic activity of this study indicated that the best species for extracting the enzyme is sorghum, although barley malt is the most common in extracting this enzyme, as well as the two types of wheat and millet, which also gave effective values. The use of sorghum cv. Kaffir in extracting the amylase enzyme recorded the highest qualitative effectiveness before and after germination compared to the Engath, Rabeh, and Geza species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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