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Physicochemical studies on starch isolated from Egyptian wheat cultivars

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ABSTRACT

In the current study, starch was isolated from two Egyptian wheat cultivars, namely, Wheat Durum BeniSuef 1 and Bread Wheat Misr 1. The isolated starches were subjected to versatile physicochemical study, comprising, proximate analyses (contents of ash, moisture, protein, total carbohydrates), total organic matter (TOM), total organic carbon (TOC), undesired elemental content (chromium, nickel, arsenic, lead and cadmium), nutritive elemental content (sodium, potassium, phosphorus, magnesium, calcium, manganese, iron, cobalt, copper, zinc and selenium), other elements (molybdenum, tin and antimony), amylose, amylopectin, pH value, water binding capacity (WBC), total hydrolysable carbohydrates, swelling power and solubility. Spectral studies including Fourier transform infrared (FT-IR) and X-Ray diffraction pattern of the isolated starches were thoroughly envisaged.

Key words: Physicochemical properties, Starch, isolation, wheat, cultivars, FT-IR, X-ray

INTRODUCTION

Since prehistoric times and throughout the recorded history till recent era wheat is one of the oldest, most extensively cultivated, and higher nutritional value crop in the world, on which different industries were based (Olsen, 1994; Yoo and Jay, 2002). Starch is the most abundant constituent of wheat grains (ca. 70–75%) that includes two types of α-D-glucose polymers: amylose and amylopectin. Amylose which is a linear molecule, consists of α-(1,4)-linked D-glucopyranosyl units having a degree of polymerization (DP) in the range of 500–6000 glucose residues. Amylose molecule has very few α-(1,6)- linkages, whereas amylopectin molecule has a highly branched structure linked with α-(1,6)-glucosidic bonds. Amylopectin has greater DP ranging from 3 × 10^5 to 3 × 10^6 glucose units (Hung *et al*., 2006; Tester *et al*., ,2004). Depending on the botanical source, amylose/amylopectin ratio in starches varies; normal wheat starches consist of 22–35% amylose and 65–78% amylopectin, but the waxy (amylose-free) wheat starches contain essentially 100% amylopectin (Cai and Shi, 2010).

Because of many factors, like being cheap natural material and the ease with which its physicochemical properties can be altered [through chemical or enzymatic modification and/or physical treatment (Jobling, 2004)], starch has been a useful material for different wide and versatile applications, like, processed food, paper, textile, chemical and pharmaceutical industries.

Starch is a major component of food products because, its pasting properties, retrogradation, and viscosity characteristics are extremely important to the appearance,

structure, and quality of foods. In addition, wheat grain starch is an important component of the fermentation process and directly affects the quality of the dough (Goesaert *et al.,* 2005; Yasui *et al.,* 2009). This is due to the fact that different amylose/amylopectin ratios and structure of starch granules affect the physicochemical properties, the processing properties of flour, and the edible quality of the end-use products (Kozlov *et al.,* 2006; Kozlov *et al.,* 2007).

Proper rate of glucose release and absorption from digesting starch may play an important role in human health by maintaining proper blood glucose levels (Zhang *et al.,* 2008). According to the rate and extent of an in vitro digestion, starch has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst and Hudson, 1996). SDS is the starch fraction that is digested completely in the small intestine at a lower rate than RDS, SDS tends to provide a sustained supply of glucose with a low glycemic index (GI) that may contribute to the control and prevention of various hyperglycaemia-related diseases (Cummings *et al.*, 1996). Besides, SDS may be beneficial to maintaining body weight when it is used as a raw material in the production of foodstuffs (Jenkins *et al.*, 2002). Hence, foods containing higher amount of SDS are regarded as functional foods with low GI (Han *et al.*, 2006; Zhang and Hamaker, 2009), and SDS has attracted more attention in recent years.

Till the moment, there is no single reported publication studying the physicochemical properties of starches isolated from different Egyptian crop cultivars. This fact, together with previously mentioned facts prompted us to isolate starches from two Egyptian cultivars, namely, Wheat Durum BeniSuef 1 and Bread Wheat Misr 1 for studying their various physicochemical properties.

MATERIALS AND METHODS

Materials:

Two cultivars of wheat, namely Wheat Durum BeniSuef 1 and Bread Wheat Misr 1, and other cultivar of corn namely Giza TWC 352 Y 352, were obtained from Field Crops Research Institute (FCRI), Agriculture Research Center, Giza, Egypt. The obtained cultivars were stored in refrigerator at 0-5 °C till the time of analysis.

Chemicals and reagents

All chemicals were purchased from Sigma, Aldrich, Merck, Fluka, Riedel-de Haën, Fisher and were of analytical grade. All chemicals and reagents used for elemental analysis were of Trace SELECT metal grade.

Starch isolation

Wheat grains were washed with tab water, and soaked in 0.16% $Na_2S_2O_5$ solution at 50℃ in oven for 24 h. The supernatant was discarded; wheat grains and corn kernels were washed two times, mixed with $Na_2S_2O_5$, blended with a blender and then passed through a 75 µm sieve. The sediment on the sieve was taken, blended with $Na_2S_2O_5$ and passed through the sieve again. The starch paste was re-suspended in 0.1% NaOH solution and left soaked in fridge for 48 h. The suspension was then centrifuged at 3431 rpm for 20 min, washed successively with 0.1% HCl followed by deionized water (each wash includes centrifugation and discarding supernatant) then methanol was added and the suspension was left soaked in methanol overnight. After discarding methanol, acetone was added twice and the mixture was separately centrifuged two successive times 3431 rpm for 20 min (including discarding the supernatant in each time). The formed starch

was collected by filtration on Whatman 1 filter paper (24 cm diameter), washed with petroleum ether and then completely dried in an oven at 40℃ for 24 h in an air oven.

Poximate analysis

1. Ash content

Determination of ash content was made as described by AOAC (2012). An empty clean crucible for each sample was placed in a muffle furnace at 600^{o}C for an hour, cooled in desiccator and then weighed (W_1). About one gram of each starch sample (W_2) was put in crucible. The sample was ashed in muffle furnace at 550 ^{o}C for at least 6-8 h. The appearances of gray white ash indicate complete oxidation of all organic matter in the sample. After ashing furnace was switch off. The crucible was cooled in a desiccator and weighed (W_3). Percent ash was calculated by following formula:

% Ash = (Difference in weight of ash/ W_2) x 100. Difference in weight of ash= W_3-W_1.

2. Fat content

Fat content was measured using filter bag technique by extracting with petroleum ether (bp 40-60 ^{o}C) using ANKOM-XT 15 Extractor (AOCS Official Method).

3. Moisture content

Moisture was determined as described by (AOAC, 2012) via oven drying method. About 1.5 g of each starch sample was accurately weighed in clean, dried crucible/or glass beaker (W_1). The crucible was allowed to dry overnight in an oven at 100-105^{o}C until a constant weight was obtained. Then the crucible/ or glass beaker was placed in a desiccator for 30 min to cool. After that it was weighed again (W_2). The percent moisture was calculated by following formula: %Moisture= 100 x (W_1-W_2)/sample weight, where, W_1 = Initial weight of crucible + Sample, W_2 = Final weight of crucible + Sample.

4. Protein Content

Protein content was quantified as described by (AOAC, 2012) using Kjeltec^{TM} 8400 analyzer unit (FOSS). A specific weight or of dried samples was taken in a kjeldahl digestion flask. The sample was digested with hot concentrated sulfuric acid containing $CuSO_4.5H_2O/K_2SO_4$ catalyst mixture. After complete digestion (color of digested material turns to blue green in color), the digest was cooled, diluted with water and subjected to distillation step. Distillation was continued for few minutes and NH_3 produced was collected as NH_4OH in a receiver containing certain volume of 4% boric acid solution with few drops of modified methyl red indicator. The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink color. A blank was also run through all steps as above. Percent crude protein content of the sample was calculated by using the following formula: % Crude Protein = 6.25 x %N.

4. Total carbohydrate content

Total carbohydrate is either measured specrophometrically using phenol sulfuric acid method reported by (Dubois *et al.,* 1956) or calculated by difference method (AOAC, 2012).

5. Total organic matter content and total organic carbon

About 10 gm of the starch was weighed dried in oven at 105^{o}C for overnight, in a pre weighed crucible. After drying the crucible and cooling in a desiccator to room temperature for ½ h, the crucible with its contents was recorded (W_1).Thereafter, the crucible with its contents was ignited in a Muffle furnace at 650 – 700^{o}C for 6-8 hrs. After cooling to

room temperature in a desiccator for ½ hr, the crucible with its contents was weighed (W_2).

Total Organic matter%= 100 x [Weight after drying (W1) – Weight after ashing]/ sample weight.

Total C% = (Total organic matter)/1.724 (AOAC, 2012; FCO, 1985).

Note; the reference time of AOAC(2012) and FCO (1985) is at least 6h for drying at 105^{o}, but the actual time for drying starch samples in the current work was overnight, to secure complete drying and constant weight.

6. Elemental content of starches

Elemental analysis was done based on dry matter. Thus, starch samples (about 5 gm) were dried in an air oven at 100℃ overnight prior to analysis. Starch samples were digested using microwave digestion technique (speed wave micro wave digestor, Berghof). About 0.2-0.5g of each kind of starch was weighed in the appropriate microwave vessel using trace metal grade concentrated nitric acid (Fisher, 67-69%). After digestion, all samples were diluted to 100 ml with deionized water. Starches were analyzed using either Graphite Technique (via ContrAA 700 atomic absorption spectrometer, Analytikjena) or inductively coupled plasma technique [via Optima 2000 DV ICP, PerkinElmer instruments), where contents of arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), selenium (Se), tin (Sn), cobalt (Co), antimony (Sb), molybdenum (Mo), copper (Cu), manganese (Mn) were quantified using graphite technique (ContrAA 700 appliaction notes) contents of calcium (Ca), iron (Fe), zinc (Zn), potassium (K), sodium (Na), phosphorus (P) and magnesium (Mg) were quantified using ICP technique (AOAC, 2012).

7. Amylose content and amylopectin

Estimation of amylose was achieved as reported by Sowbhagya and Bhattacharya (1971). Each starch sample was defatted using petroleum ether (bp 40–60 °C) using using ANKOM-XT 15 Extractor. In a 50 mL conical flask, about 100 mg (on dry matter basis) of defatted starch sample and to it was added 1 mL of ethyl alcohol, followed by 10 mL of 1 N sodium hydroxide and the mixture was kept overnight at room temperature. The next day, sample was boiled in a water bath at 100 °C for 15 min, cooled and then transferred quantitatively to 100 mL conical flask and finally made up to volume with deionized water. An aliquot v (2.5-5 mL) of the dispersion was accurately pipetted out into a 100 mL conical flask followed by successive addition of 50 mL of water and 1 mL of 1 N acetic acid. After shaking the whole mixture, 2 mL of 0.2% iodine in 2% potassium iodide solution was added and the volume was made up to mark with deionized water, and kept at 30 °C for 20 min. Absorbance was read at 620 nm in a UV-visible spectrophotometer (model Specord-200 analytikjena) with a blank (without sample). Standard amylose (Sigma, Germany), was prepared by dissolving in a 100 mL conical flask 100 mg amylose powder in 1 mL ethyl alcohol and 10 mL1N sodium hydroxide, covered and heated in a water bath at 100 °C for 2 min. The solution was cooled at room temperature and 1 mL was accurately pipetted to which 50 mL double deionized water, 1 mL of 1 N acetic acid and 2 mL of 0.2% iodine in 2% potassium iodide solution were added, made up to volume with deionized water, and kept at 30 °C for 20 min. The color was also read at 620 nm.

The total amylose composition was calculated using the formula of Sowbhagya and Bhattacharya (1971): Amylose content (% dry basis) = (R x a x 1 x 100)/(A x r x v) where:

R = absorbance of sample, A = absorbance of standard amylose, a = weight of standard amylose (mg) in 100 mL, r =weight of sample taken (mg) in 100 mL and v is the aliquot volume (mentioned above, 2.5-5 mL, taken from the 50 mL solution and diluted to 100 mL solution).

Amylopectin was calculated by difference of total carbohydrate content and amylose content.

8. pH Value of the isolated starches:

It was measured in starch slurries of 25 g starch in 50 ml distilled water as described by AOAC (2012) and FCO, (1985). pH value was measured by ORION 5 STAR multifunction (pH. ISE. COND. DO) benchtop meter (Thermo Electron Corporation)

9. Water binding capacity measurement

WBC of all starches was determined using method described by Yamazaki (1953) and Medcalf and Gilles (1965). with some modifications. A suspension of 2.5g from each starch (dry weight) in 37.5 ml deionized water was agitated for 1 h on a shaker and centrifuged at 5430 rpm for 10 min (equivalent to 3000g using Sigma centrifuge). The supernatant (free water) was removed from the wet starch, which was drained for 10 min, and the wet starch was weighed.

10. Total hydrolysable carbohydrates

Sampleswere measured as previously reported by Dubois *et al.*(1956). Each sample was prepared by dispersing about 100 mg (dry based matter) of each starch sample in 1 mL of ethyl alcohol in a 100 mL conical flask (previously washed successively with concentrated boiled nitric acid then deionized water, followed by drying at 100^{o}C overnight), slowly stirred, treated with 9 mL of 1 N sodium hydroxide, and heated in boiling water bath for 10 min with continuous shaking. The sample was cooled to room temperature and transferred quantitatively to a 100 mL volumetric flask, and made up to volume with deionized water. Accurately pipetted 5 mL of this solution was neutralized using 0.1 N HCl and phenolphthalein indicator. To an aliquot volume of the sample was added, 1 mL of 5% phenol followed by 5 mL concentrated H_2SO_4 cautiously on the side of the test tube. After cooling the test tubes to room temperature for 30 min and the absorbance was read at 490 nm using a UV-spectrophotometer (mode Specord-200 analytikjena) (with a blank (without sample). Standard glucose was treated the same way as the samples. Through the software of the instrument, linear regression model was applied to develop the prediction equation that was used for determining the glucose concentration of the samples.

11. Swelling Power and Solubility

Were determined as previously reported by Schoch (1964) and Unnikrishnan and Bhattacharya (1981) with some modifications as follow: A suspension of about 1g of each isolated starch (dry weight) in about 40 mL deionized water was incubated in water bath at each of the investigated temperature, separately (30 ^{o}C/ 60 ^{o}C / 95 ^{o}C) for 30 min. They were then centrifuged at 8300 rpm for 20 min. Supernatant was decanted carefully and kept for measuring solubility, and the residue was weighed for swelling power determination. An aliquot volume of the supernatant was pipetted out to an empty preweighed clean and dry beaker that was kept overnight in an air oven. After complete evaporation of the contents of the beaker, it was dried in an air oven at 100-105^{o}C overnight.

Swelling power = (Wt of the wet residue− Wt of the dried sample)/Wt of sample on dry base

Solubility = [(Wt of the dry residue from) (100/Aliqot volume)(100)]/Starch weight.

12. Fourier transform infrared (FT-IR) spectroscopy

The change in chemical structure of the starch was qualitatively analyzed by using FT-IR (Spectrum Two, PerkinElmer FTIR spectrometer). Samples were prepared by grinding the finely powdered starch with KBr. The spectrum was recorded over the wave number range between 400 and 4000 cm^{-1}. The starch samples were dried at 105^{o}C h before analysis to avoid interference by moisture and kept in a desiccator to be cooled to room temperature before its scanning.

13. X-Ray diffraction pattern

The X-ray patterns of the dry isolated starches were done at the Nano and Advanced Material Central Lab (NAMCL), Agriculture Research Center, Giza, Egypt using an X-ray diffractometer (X' Pert Pro PANanalytical, Netherland) operated at 30 mA and 40 kV. The scanning region of the diffraction angle (2ɵ) was from 40 to 80 with scan step time 0.5 second. Anode material is copper with k-α1= 1.54060 and k-α2= 1.54443.

RESULTS AND DISCUSSION

1. Physicochemical properties of isolated starches

1.1. Proximate analysis of isolated starches

Table (1) briefs the proximate analysis of isolated starches from the studied wheat cultivars Durum BeniSuef1 and wheat Bread Wheat Misr1. It is clear from Table (1) that cultivar variation affects the chemical composition of the isolated starches. Thus, cultivar Durum BeniSuef 1 recorded higher ash (0.026%) and protein (3.72%) contents when compared to cultivar Bread Wheat Misr 1 which recorded 0.01% and 0.55%, respectively. On the other hand, cultivar Bread Wheat Misr 1 recorded higher fat (0.79%), moisture (9.96%) and total carbohydrate (88.96%) than cultivar *Durum BeniSuef 1* that recorded 0.64%, 8.86% and 86.75%, respectively.

Table (1). Composition (%) of isolated starches from wheat cultivars

Wheat cultivar	Ash	Fat	Moisture	Protein	Total Carbohydrate
Durum BeniSuef 1	0.026	0.64	8.86	3.72	86.75
Bread Wheat Misr 1	0.01	0.79	9.69	0.55	88.96

The variation in chemical composition was noted by **Kuar *et al.* (2007**) who reported ash content in the range of 0.06 to 0.45% for different potato cultivars grown at different locations.

1.2. Total organic matter of isolated starches

Table (2) shows the total organic matter content (TOM) as well total organic carbon content of the isolated starches. From table 2 it is clear that cultivar Durum BeniSuef 1 recorded higher total organic matter (91.12%) as well as total organic carbon content (52.85%) when compared to cultivar Bread Wheat Misr 1which recorded 90.30% and 52.38%, respectively. These results support those obtained in table 1, as it is well known that the major organic matter in starch include fat, protein and carbohydrate.

Table (2). Total organic matter (TOM) and total organic carbon (TOC) contents of isolated starches (%)

Wheat cultivar	Total Organic matter (TOM)	Total organic carbon (TOC)
Durum BeniSuef 1	91.12	52.85
Bread Wheat Misr 1	90.30	52.38

1.3. Concentration ofundesired elements in isolated starches

Table (3) illustrates the concentration of some undesired elements in starch as they are reported to be undesirable in animal feed (DIRECTIVE 2002/32/EC). Chromium (Cr), nickel (Ni), arsenic (As), lead (Pb) and cadmium (Cd) were measured using the graphite technique in ppb concentration unit (1ppb = 0.001 ppm). Arsenicsignal (at λ = 193 nm), when measured using the high resolution continuum source (HR-CS) is sometimes interfered with PO molecular signal, coming from reaction of phosphorus in the sample and nitric acid. Thus, in Table 3, the concentration of arsenic was measured using two different techniques, the first is the direct measurement technique, while the second techniques measurement after applying the recommended PO correction model (As^{a}, $As^{a,b}$ in Table 3). The difference between the two measurements assured the existence of PO molecular interfering signal at the same wave length of arsenic. So, the accurate results were obtained after compensation of the direct overlap of element and PO molecular absorption lines and performing the spectrum correction as reported (ContrAA300/600/700).As in arsenic measurement, cadmium can suffer PO molecular signal overlap with cadmium at measuring line λ 228 nm. Thus both measurements with and without PO correction model are shown in Table 3. There is no much great difference between the two measurements Cd^{a}, $Cd^{a,b}$.

The presented data in Table (3) reveals that starch isolated from cultivar *Durum BeniSuef 1* recorded higher contents of chromium (Cr; 1196 ppb), arsenic (As^{a}; 998.3 ppb/ $As^{a,b}$; 974 ppb), lead (Pb; 1402 ppb) and cadmium (Cd^{a} ; 995.8 ppb/ $Cd^{a,b}$; 995.5 ppb) when compared to starch isolated from cultivar Bread Wheat Misr 1 which recorded 1090 ppb, 422.6 ppb/ 359.2 ppb, 1017 ppb and 992.7 ppb/992.6 ppb, for the same elements, respectively. On the other hand, cultivar Bread Wheat Misr 1 recorded higher nickel content (3270 ppb) compared to cultivar

Table (3). Quantitative analysis of undesired elements (ppb).

Wheat cultivar	Cr^{a}	Ni^{a}	As^{a}	$As^{a,b}$	Pb^{a}	Cd^{a}	$Cd^{a,b}$
Durum BeniSuef 1	1196	3076	998.3	974	1402	995.8	995.5
Bread Wheat Misr 1	1090	3270	422.6	359.2	1017	992.7	992.6

[a] Measured with graphite technique

[b] Measured using PO correction model

1.4. Concentration ofsome nutritive and other elements in isolated starches

Table (4) Shows the concentration of some nutritive elements which have a specific biochemical function in humans, animals and plants beside other elements. Of the useful nutritive elements sodium (Na), potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), Manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and Selenium (Se) and molybdenum (Mo). Cobalt (Co), is available for use by animals only after having been processed into complex molecules (e.g., vitamin B12) by bacteria. Other elements, like Tin (Sn) and antimony concentrations (Sb) are also shown in Table 4.Copper (Cu) concentration was measured by two graphite techniques, one of them is the direct graphite measurement (Cu^{a})and the other is measurement after compensation of interfering PO signal via correction model ($Cu^{a,b}$) which is more precise and of best accuracy.

Table (4). Concentration of some nutritive elements and other elements in isolated starches.

Wheat cultivar	Na^{c} (ppm)	K^{c} (ppm)	P^{d} (ppm)	Mg^{c} (ppm)	Ca^{c} (ppm)	Mn^{a} (ppb)	Fe^{c} (ppm)	Co^{a} (ppb)	Cu^{a} (ppb)	$Cu^{a,b}$ (ppb)	Zn^{c} (ppm)	Se^{a} (ppb)	Mo^{a} (ppb)	Sn^{a} (ppb)	Sb^{a} (ppb)
Durum BeniSuef1	32.66	1.222	19.29	75.39	63.97	74.2	3.375	246.4	10210	10180	104.0	1540	15150	992.7	978.7
Bread Wheat Misr 1	79.80	4.107	16.00	52.41	39.02	31.0	1.041	271.1	410.5	380.8	91.23	1053	15570	781.9	1040

[a] Measured with graphite technique
[b] Measured using PO correction model
[c]Measured using ICP technique
[d]Measured using molybdovanadatespectroohotometric method

Data in Table (4) reveal that cultivar *Durum BeniSuef 1* recorded higher contents of phosphorous (P), magnesium (Mg), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu^{a}/ $Cu^{a,b}$), zinc (Zn),selenium (Se) and tin (Sn), being, 19.29 ppm, 75.39 ppm, 63.97 ppm,74.2 ppb, 3.375 ppm, 10210 ppb/10180ppb, 104.0 ppm, 1540 ppb ppb and 992.7 ppb, compared to cultivar Bread Wheat Misr 1 which recorded, 16.0 ppm, 52.41 ppm, 39.02 ppm, 31.0 ppb, 1.041 ppm, 410.5 ppb/ 380.8 ppb, 91.23 ppm, 1053 ppb and 781.9 ppb, for the same elements, respectively. On the other hand, cultivar Bread Wheat Misr 1 recorded higher contents of sodium (Na), potassium (K), cobalt (Co), molybdenum (Mo) and antimony (Sb), being, 79.80 ppm, 4.107 ppm, 271.1 ppb, 15570 ppb and 1040 ppb, compared to cultivar Durum BeniSuef1, which recorded, 32.66 ppm, 1.222 ppm, 246.4 ppb, 15150 ppb and 978.7 ppb, for the same elements, respectively.

Phosphorous which is an important element that could affect functional properties of starches, was reported to be linked in some crops, like potato, in the form of phosphate ester groups (Whistler and Be Miller, 1997; Takeda *et al.*, 1986).The variation in element concentrations between the studied wheat cultivars is consistent to those reported by Pe´rez*et al.* (2005) as they reported different concentrations of phosphorous content in *Colocasia esculenta* [0.01 mg/100g, (equivalent to0.1 ppm)], *Xanthosoma sagittifolium* [0.07 mg/100 g (equivalent to0.7 ppm)] and *Manihot esculenta* [0.05 mg/100 g (equivalent to0.5 ppm)].

1.5. Amylose and Amylopectin in Isolated Starches

Starch is a mixture of two main components: amylose and amylopectin. Amylose is essentially a linear polymer consisting of glucose units linked by α-(1→4) glycosidic bonds, slightly branched by α-(1→6) linkages. Amylopectin is a highly branched polymer constituted of

relatively short branches of α-D-(1→4) glycopyranose that are interlinked by α-D-(1→6) glycosidic linkages (Dufresne 2014). Starch contains approximately 20-30% of amylose which is more soluble in water than the other component amylopectin (70-80%) (Brown and Poon, 2005). Because of its tightly packed helical structure, amylose is more resistant to digestion than other starch molecules and is therefore an important form of resistant starch. The number of repeated glucose subunits (n) is usually in the range of 300 to 3000, but can be many thousands (Wikipedia;https://en.wikipedia.org/wiki/Amylose).

Table (5) shows the amylose amylopectin pattern of the isolated starches from the studied wheat cultivars. Cultivar Durum BeniSuef 1 recorded higher amylose content (59.84%) and lower amylopectin content compared to Cultivar Bread Wheat Misr 1 which recorded 46.51% for amylose and 42.45% for amylopectin.

The obtained results for the studied cultivars reveals that both cultivars have an amylose-amylopectin profile moderate between normal wheats and waxy ones. The ratio of amylose to amylopectin content in starch varies was reported to be depended on botanical source; normal wheat starches consist of 22–35% amylose and 65–78% amylopectin, but the waxy (amylose-free) wheat starches contain essentially 100% amylopectin (CaiandShi, 2010).

Table (5). Amylose and amylopectin contents in isolated starches.

Wheat cultivar	Amylose (%)	Amylopectin (%)
Durum BeniSuef 1	59.84	26.91
Bread Wheat Misr 1	46.51	42.45

In biochemical transformations through the plant, and after the production of ADP-glucose by ADP-glucose pyrophosphorylase (AGPase), the "waxy" proteins (granule bound starch synthase I) encoded by the Wx genes are solely responsible for amylose synthesis (reviewed inTetlow, 2006). In contrast, amylopectin synthesis involves a host of enzymes such as starch synthases (SS) I, II, III, IV, starch branching enzymes (SBE) I and II, and starch de-branching enzymes (reviewed in Tetlow, 2006).Several starch biosynthetic proteins remain bound to the interior of starch granules with a subset of these proteins designated the starch granule proteins (SGPs). Using SDS-PAGE, Yamamori and Endo (1996) separated the SGPs from bread wheat starch into SGP-1, SGP-2, SGP-3 and WX. The SGP-1 fraction was further resolvedinto SGP-A1, SGP-B1, and SGP-D1 and the associated genes localized to the homoeologous group 7 chromosomes (Yamamori and Endo, 1996). SGP-1 proteins are isoforms of SSII encoded by the genes SSIIa-A, SSIIa-B, SSIIa-D on the short arms of group 7 chromosomes (Li *et al*., 1999).A survey of hexaploid wheat germ plasm identified lines lacking SGP-A1, SGP-B1, or SGP-D1 (Yamamori and Endo, 1996) which were crossed to create an SGP-1 null (Yamamori *et al.*, 2000). The SGP-1 null had a 29% increase in amylose content (37.3% null vs. 28.9% wild-type), deformed starch granules, reduced starch content, and reduced binding of SGP-2 and SGP-3 to starch granules. These SGP-1 mutations were later shown to reduce starch binding without impacting SGP protein expression levels (Kosar-Hashemi *et al.*, 2007). Lafiandra *et al.* (2010) reported that SGP-1 null lines created

from crosses between the Durum (*Triticum turgidum ssp.* Durum) cultivar 'Svevo' and hexaploid SGP-A1/B1 null lines (Yamamori and Endo, 1996) had an 89% increase in amylose content compared to Svevo (43.6% SGP-1 null vs 23% wild-type) as well as reduced binding of SGP-2 and SGP-3. Elimination of *SbeIIa* in Durum through RNA interference also resulted in increased amylase ranging from + 29% to + 200% (24% wild-type vs. 31-75% *SbeIIa* RNAi lines) (Sestili *et al.,* 2010). The very high amylose results observed by Sestili *et al.* (2010) may not be due solely to *SbeIIa* expression reduction since *SbeIIa* mutants have amylose level increases similar to those of SSIIa mutations (28% sbeIIa versus 23% wild-type) (Hazard *et al*., 2012).

1.6. pH of the isolated starches

pH values of the isolated starches were found to be acidic (less than pH 7) recording 3.11 and 3.45 for cultivars Durum BeniSuef 1and Bread Wheat Misr 1, respectively (Table 6). This is consistent to reported data of pH of different starches separated from crops at different locations (Kaur *et al.,* 2007).

Table (6). pH of the isolated starches.

Wheat cultivar	pH
Durum BeniSuef 1	3.11
Wheat (*Bread Wheat Misr 1*	3.45

1.7. Hydrolysable effect of short time alkali treatment on the isolated starches

The isolated starches were heated at reflux temperature in diluted ethanolic sodium hydroxide solution for 15 minutes. It is clear from the Table (7) that both cultivars have close hydrolysable and resistant starch content. Thus, cultivar Durum BeniSuef 1 recorded slightly lower hydrolysable starch content and higher resistant starch content being 64.58% and 65.09%, compared to cultivar Bread Wheat Misr 1 that recorded 22.17% and 23.87%, for the same analytes, respectively.

Table (7). Effect of ca. 0.9 M sodium hydroxide on the amount of hydrolysable carbohydrate.

Wheat cultivar	Hydrolysable Starch (%)	Resistant Starch (RS) (%)	RS % to Total Starch (%)
Durum BeniSuef 1	64.58	22.17	25.56
Bread Wheat Misr 1)	65.09	23.87	26.83

1.8. Water binding capacity of the isolated starches

The water binding capacity (WBC) of the isolated starched (Table 8) reveals that cultivar *Durum BeniSuef 1* recorded the higher value being 123.67%, compared to cultivar *Bread Wheat Misr 1,* which recorded 62.60%.

Sandhu *et al.* (2004) reported a range of96% to 107%forWBC of starches isolated from different corn types where this difference was attributed to the variation in their granule structure. The differences in degrees of availability of water binding sites among the starches may have also contributed to the variation in WBC (Wotton & Bamunuarachchi, 1978).

Table (8). Water binding capacity of the isolated starches

Wheat cultivar	Water Biding Capacity (WBC) (%)
Durum BeniSuef 1	123.67
Bread Wheat Misr 1	62.60

1.9. Solubility of the Isolated Starched and Temperature Effect on It

Solubility of the isolated starches (Table 9) was studied at three different temperatures 30°C, 60°C and 95°C, respectively. At 30°C and 60°C, cultivar *Durum BeniSuef 1*, recorded the higher solubilities being 2.04%, 12.79%, compared to cultivar Bread Wheat Misr 1, which recorded, 0.76% and 9.96%, at the same temperatures, respectively. On the other hand, at 100 °C, cultivar *Bread Wheat Misr 1* recorded the higher solubility being 100%, compared to, cultivar Durum BeniSuef 1, which recorded 73.85%, at the same temperature.

Table (9). Solubility (%) of isolated starches.

Wheat cultivar	Solubility (%) at 30°C	Solubility (%) at 60°C	Solubility (%) at 95°C
Durum BeniSuef 1	2.04	12.79	73.85
Bread Wheat Misr 1	0.76	9.96	100

1.10. Swelling power of the isolated starched and temperature effect on it

The swelling power (g/g) of the isolated starches from the studied cultivars was studied at three different temperatures as shown in Table (10). Thus, at all the studied temperatures; 30°C, 60°C and 95°C, starch isolated from cultivar *Durum BeniSuef 1*, recorded the higher swelling power, being 6.60g/g, 9.95g/g and 14.5g/g, compared to starch isolated from cultivar *Durum BeniSuef 1*, which recorded, 6.07 g/g, 9.87 g/g and 10.31 g/g, respectively.

Table (10). Swelling power (g/g) of isolated starches.

Wheat cultivar	Swelling power (g/g) at 30℃	Swelling power (g/g) at 60˚C	Swelling power (g/g) at 95˚C
Durum BeniSuef 1	6.60	9.95	14.50
Bread Wheat Misr1	6.07	9.87	10.31

Studying the swelling power in comparison with the water binding capacity led to interesting finding. The wheat cultivar, *Durum BeniSuef 1*, of higher water binding capacity (123.67%), recorded the higher swelling power, under all studied heat temperatures.

Thus, it was concluded that swelling power of starch depends on water holding capacity of starch molecules via hydrogen bonding consistent to that published by Lee and Osman (1991). Hydrogen bonds stabilizes the structure of the double helices in crystallites are broken during gelatinization and are replaced by the

hydrogen bonds with water, and swelling is regulated by the crystallinity of the starch (Tester and Karkalas, 1996).

1.11. Fourier Transform Infrared (FT-IR) Spectroscopy of Isolated Starches

Figures (1a,b & 2 a,b) and Tables (11 & 12) indicated the FT-IR spectral data of the isolated starches in comparison with that of amylose (Fig.3a,b) in the frequency region 4000-400 cm^{-1}. Cultivar *Durum BeniSuef 1* (Figs. 1a,b, Table 11), recorded characteristic bands at; 3370 cm^{-1} (OH band), 2931cm^{-1} (CH stretching vibrations), 1649 cm^{-}1 (due totightly bound water present in the starch granule),1156, 1081, 1020, 930cm^{-1} (C-Obondstretching vibrations in the finger print region). On the other hand, cultivar Bread *Wheat Misr1*(Figs. 2a,b, Table 12), recorded similar bands at 3365 cm^{-1}(OH band), 2932cm^{-1}(CH stretching vibrations), 1647 cm^{-1} (due totightly bound water present in the starch granule), 1152, 1080, 1029, 928cm^{-1} (C-Obondstretching vibrations in the finger print region).The obtained spectral data are consistent to those reported for native and modified potato starch (Fang *et al.*, 2004; Hui *et al.*, 2009). The great similarity between the spectra of the studied cultivars and amylose may assure the high levels of amylose in both cultivars (Fig. 3a). The difference in spectral data between the studied cultivars and amylose appears in the frequency regions of 500-400 cm^{-1} (Fig. 3b).

The FT-IR spectrum of starch is sensitive to changes in structure of a short-range molecular level (double helices). The absorbance bands at 1022 and 1047 $cm^{-1}$are characteristics of amorphous and ordered structures in starch (Van Soest *et al*. 1995). Thus, the ratio of 1047 cm^{-1}/1022cm^{-1} can be used to characterize the short-range molecular order of double helices in starches (Van Soest *et al*., 1995; Capron *et al*., 2007). Figures (4,5) show the absorbance bands at 1047 and 1022 cm^{-1} of starches isolated fromcultivars *Durum BeniSuef 1* and which confirms different amorphous and ordered structures between them.

Table (11) Infrared spectroscopy transmittance (%T) at different frequencies (Cm^{-1}) of wheat (*Durum BeniSuef 1*).

Peak number	X (Frequency; cm^{-1})	Y (%T)
1	3369.52	19.33
2	2931.1	40.39
3	2151.63	82.16
4	1649.38	43.74
5	1416.34	42.42
6	1369.75	41.6
7	1240.91	47.72
8	1156.23	20.25
9	1080.71	17.56
10	1019.63	11.06
11	929.72	50.67
12	860.82	61.06
13	764.27	49.69
14	708.54	45.75
15	575.14	33.73

Table (11) Infrared spectroscopy transmittance (%T) at different frequencies (Cm^{-1}) of wheat (*Durum BeniSuef 1*).		
16	526.93	36.97
17	499.17	38.79
18	487.62	41.31
19	480.06	28.55
20	471.74	21.77
21	460.97	69.21
22	450.84	29.22
23	444.86	65.78
24	436.86	73.14
25	427.93	74.28
26	421.6	86.73
27	408.17	74.96
28	403.97	67.85

Table (12) Infrared spectroscopy transmittance (%T) at different frequencies (Cm^{-1}) of wheat (Bread Wheat Misr 1)

Peak Number	X (cm^{-1})	Y (%T)
1	3365.28	0.25
2	2931.55	2.07
3	2150.79	32.36
4	1647.06	6.43
5	1458.88	3.55
6	1416.35	2.98
7	1368.66	2.5
8	1242.2	4.51
9	1151.85	0.28
10	1080.17	0.15
11	1028.5	0.05
12	1006	0.05
13	928.26	4.54
14	860.83	8.53
15	763.85	4.33
16	708.44	3.35
17	672.78	3.97
18	667.37	3.93
19	656.43	3.53
20	640.44	3.04
21	626.39	2.54
22	618	2.16
23	611	2.16
24	605.32	2.13
25	590.73	1.47

Table (12) Infrared spectroscopy transmittance (%T) at different frequencies (Cm^{-1}) of wheat (Bread Wheat Misr 1)

Peak Number	X (cm^{-1})	Y (%T)
26	585.2	1.43
27	573.86	0.69
28	570.29	0.32
29	555.8	1.04
30	549.37	1.19
31	538.36	0.36
32	534.62	0.61
33	529.05	2.36
34	523.75	2.4
35	520.17	2.37
36	515.86	1.26
37	511.02	1.24
38	503.28	4.65
39	496.98	3.97
40	492.25	1.74
41	479.28	16.55
42	474.15	22.43
43	466.05	79.02
44	457.91	136.9
45	453.52	203.47
46	449.51	80.18
47	446.07	178.88
48	438.08	316.07
49	434.32	341.79
50	429.93	308.25
51	422.92	67.24
52	419.78	36.74
53	418.22	29.68
54	413.39	205.9
55	410.01	426.51
56	406	495.55
57	401	191.67

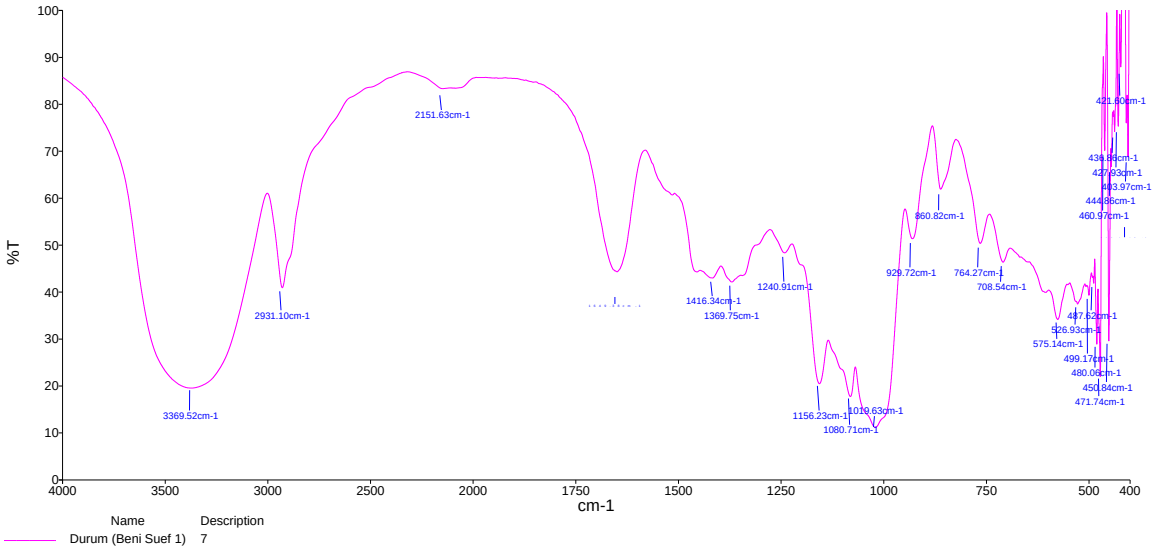


Fig. (1a) . FT-IR spectrum of wheat (*Durum BeniSuef 1*) starch in the region 4000-400 cm^{-1}

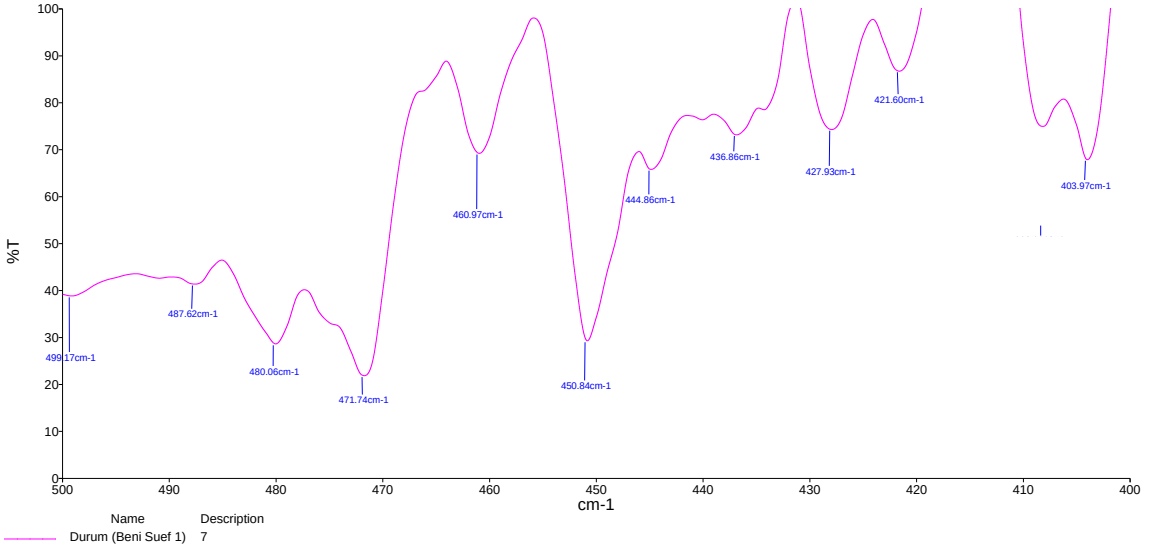


Fig. (1b). FT-IR spectrum of wheat (*Durum BeniSuef 1*) starch in the region 500-400 cm^{-1}

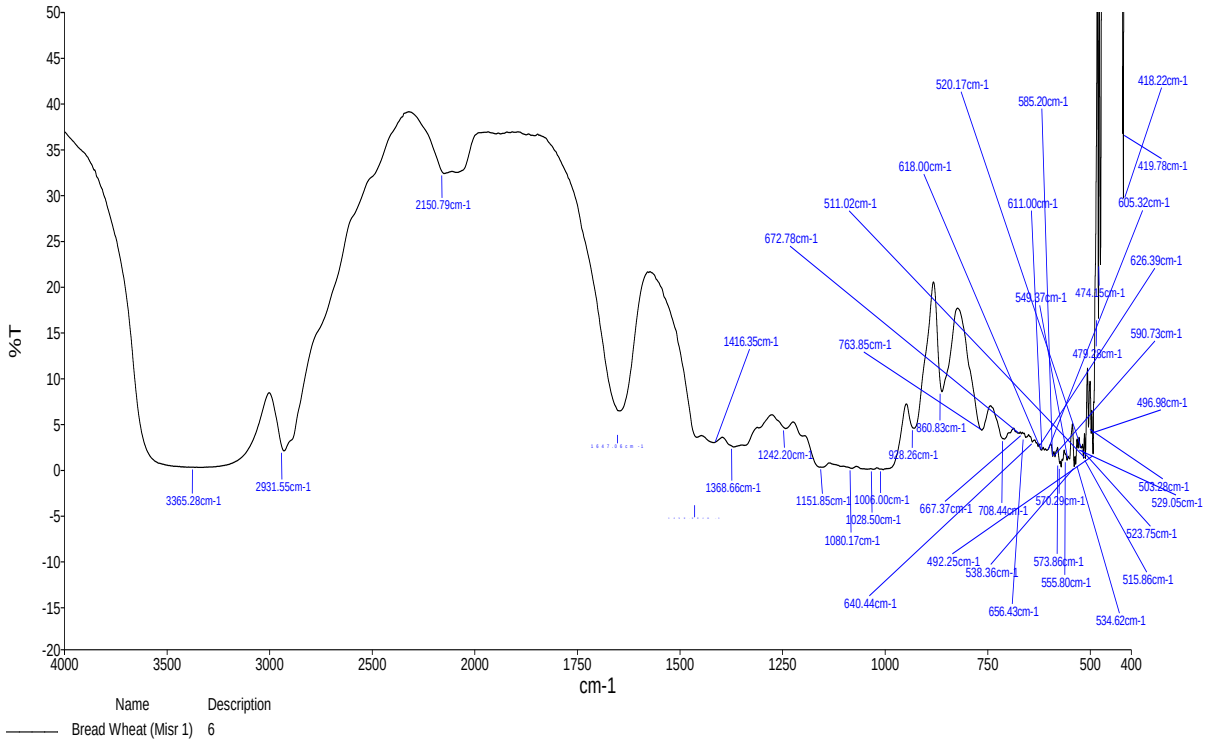


Fig. (2a). FT-IR spectrum of wheat (*Bread Wheat Misr 1*) starch in the region 4000-400 cm^{-1}

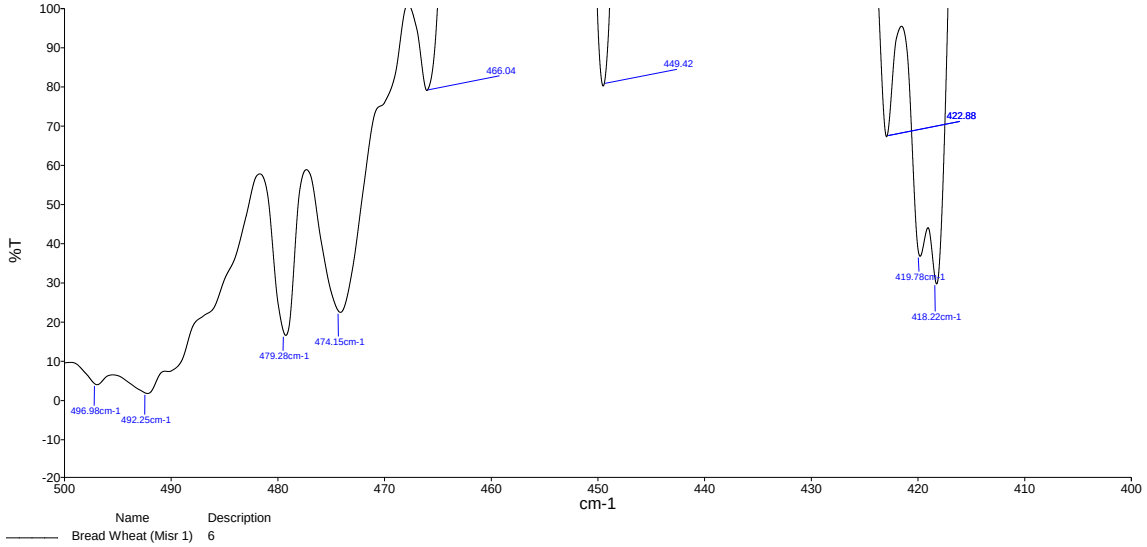


Fig. (2b) FT-IR spectrum of wheat (*Bread Wheat Misr 1*) starch in the region 500-400 cm^{-1}

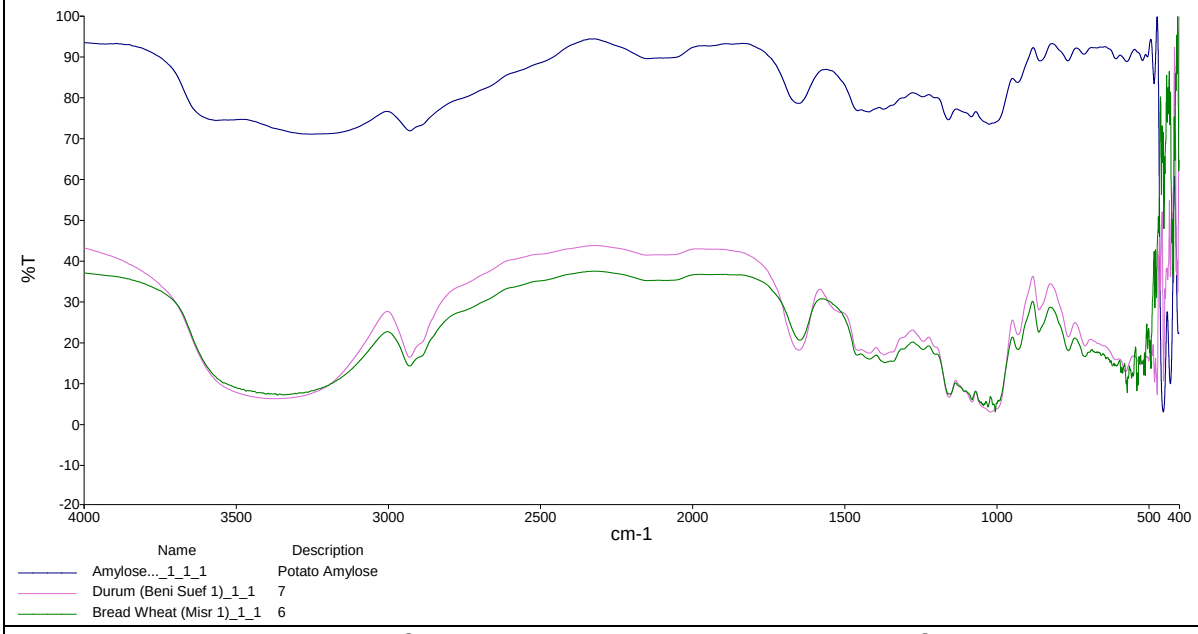


Fig. (3a). Overlaid spectra of potato amylose andstarches isolated fromcultivars*Durum BeniSuef 1* and *Bread Wheat Misr 1* in the region 4000-400 cm^{-1}

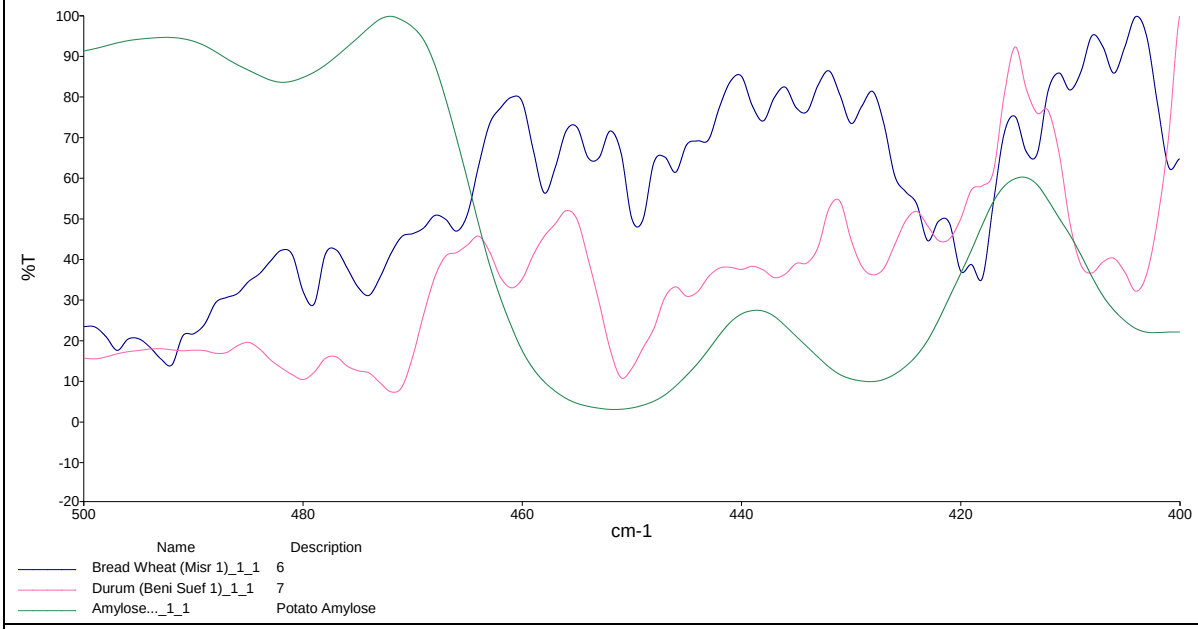


Fig. (3b). Overlaid spectra of potato amylose andstarches isolated from cultivars *Durum BeniSuef 1* and *Bread Wheat Misr 1* in the region 500-400 cm^{-1}

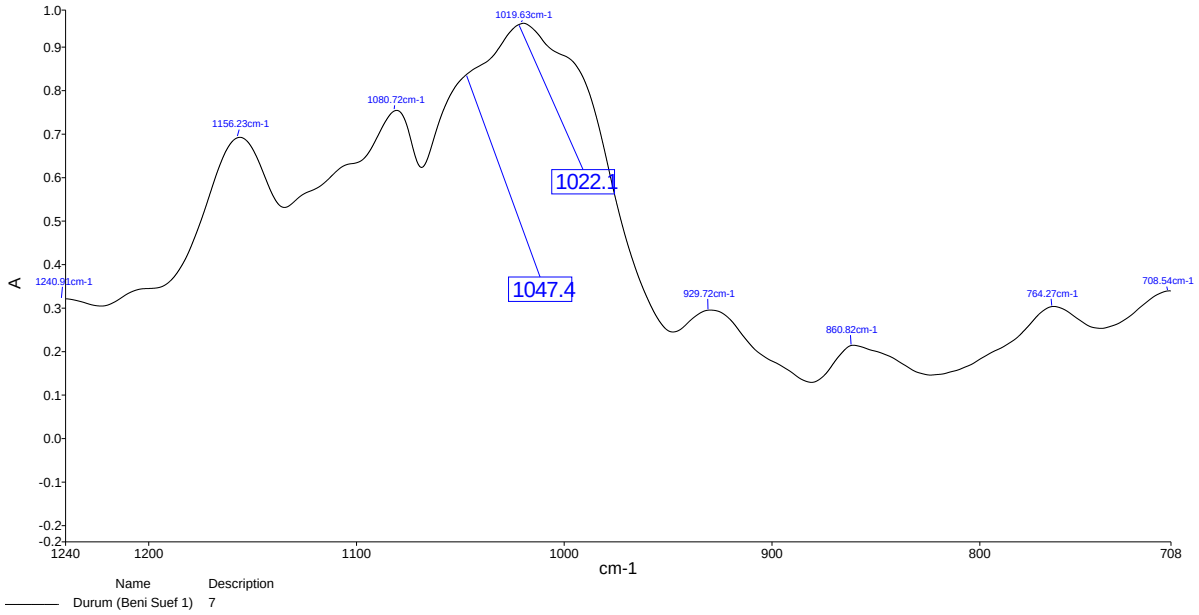


Fig. (4) FT-IR absorbance bands of starch isolated from cultivar *Durum BeniSuef 1* at 1047 and 1022 cm^{-1}

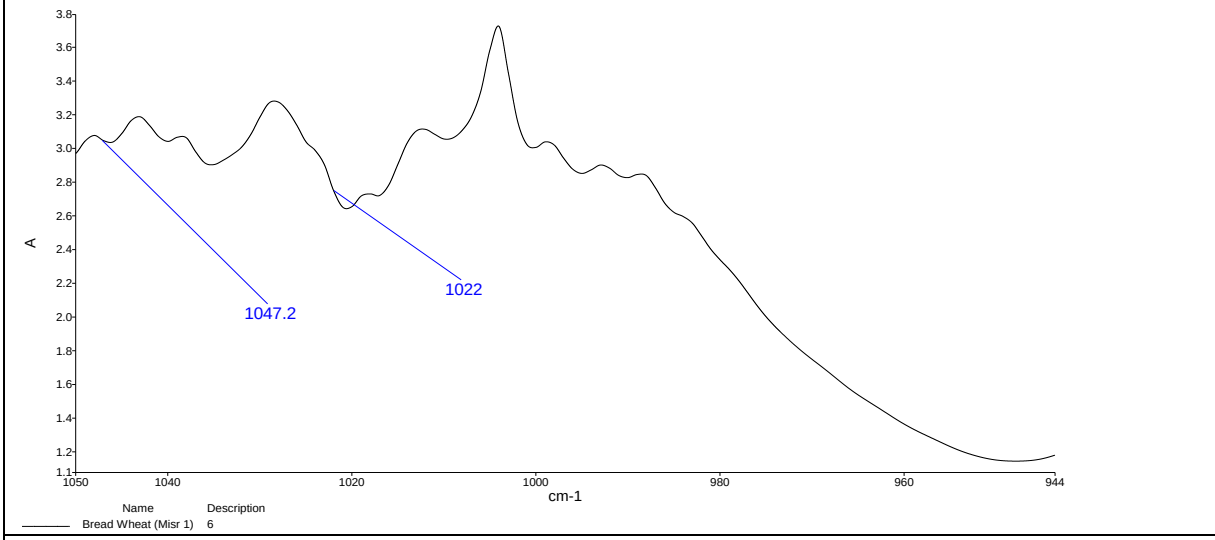


Fig. (5) FT-IR absorbance bands of starch isolated from cultivar *Bread Wheat Misr 1* at 1047 and 1022 cm^{-1}

1.12. X-Ray diffraction pattern of isolated starches:

The X-ray diffraction pattern of the crystal structure of the isolated starches are summarized in Figures (6a,b) and Tables (13, 14). Starch isolated from cultivar Durum BeniSuef1 exhibited single peaks at 2θ = 14.86^{o}, 17.05 ^{o}, 18.29 ^{o}, 23.38 ^{o}, while starch isolated from cultivar Bread Wheat Misr 1 revealed similar peaks at2θ = 15.17^{o}, 17.07 ^{o}, 18.17 ^{o}, 23.37 ^{o}, showing the typical characteristics of an A-type diffraction

pattern. Similar close diffraction pattern of A-type was previously reported (Zhang *et al.*, 2013).

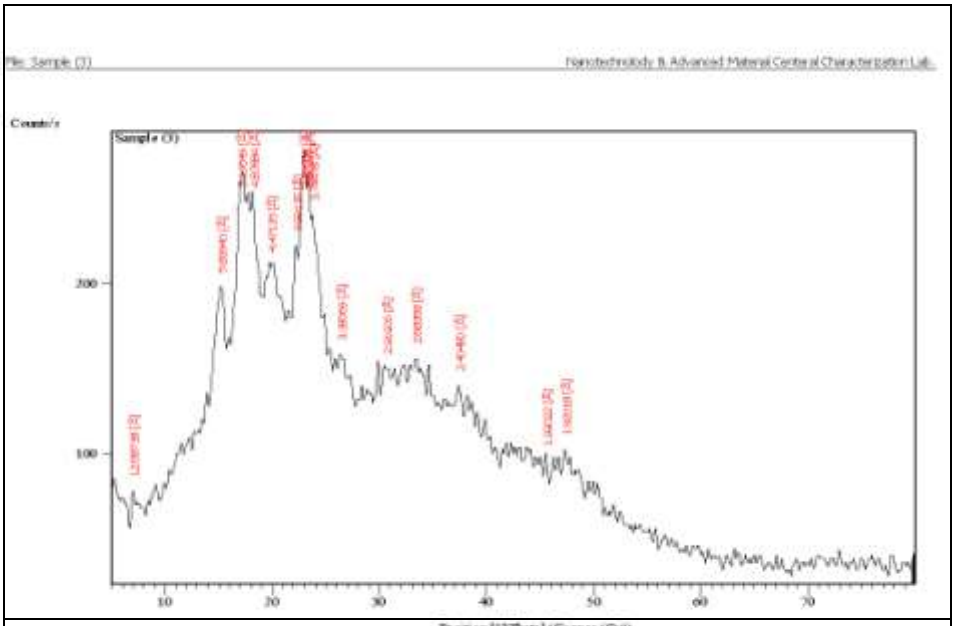
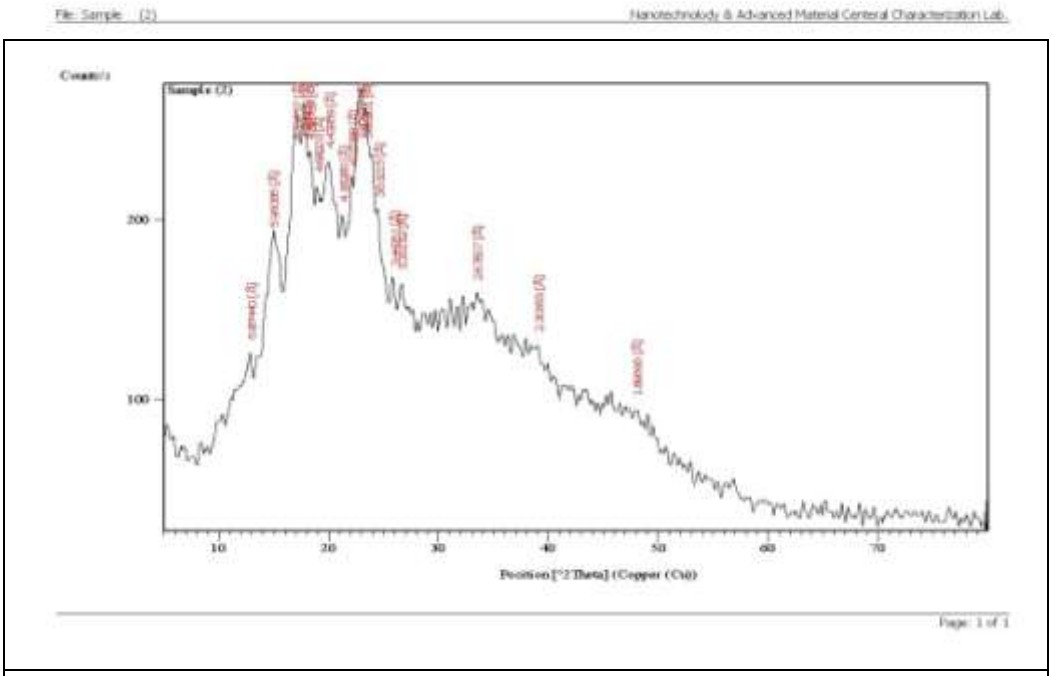


Fig. (6b). X-Ray diffraction pattern of wheat (*Bread Wheat Misr 1*) starch



Fig, (6a). X-Ray diffraction pattern of wheat (Durum BeniSuef 1) starch

Table 14. X-ray diffraction data of starch isolated from cultivar Bread Wheat Misr 1

No.	Pos. [°2Th.]	d-spacing [Å]	Height [cts]	Height [cps]	Rel. Int. [%]
1	6.9616	12.68738	3.63	7.25	7.9
2	15.1731	5.8394	23.88	47.75	52.02
3	17.0668	5.19549	35.47	70.94	77.28
4	18.1684	4.87884	22.28	44.55	48.54
5	19.8401	4.47135	2.59	5.18	5.65
6	22.2549	3.99135	18.85	37.7	41.07
7	22.9472	3.87568	45.9	91.79	100
8	23.3694	3.80347	38.32	76.63	83.48
9	23.7409	3.74478	25.8	51.61	56.22
10	26.3414	3.38069	1.98	3.95	4.3
11	30.6015	2.91906	1.31	2.62	2.85
12	33.3618	2.68358	2.45	4.89	5.33
13	37.3642	2.4048	4.93	9.86	10.74
14	45.5809	1.99022	2.66	5.32	5.8
15	47.3014	1.92018	4.7	9.41	10.25

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دراسات فيزيوكيميائية على النشويات المعزولة من أصناف القمح المصرية

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3 - قسم بحوث النسيج، المركز القومي للبحوث بالدقي

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المستخلص

في الدراسة الحالية ، تم عزل النشا من صنفين من القمح المصري هما القمح ديورم بني سويف 1 و خبز القمح مصر 1، وقد تم الحصول عليهم من معهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية، الجيزة، مصر. وقد تعرضت النشويات المعزولة لدراسات فيزيوكيميائية متعددة ، تضمنت تحليلات تقريبية (محتوي الرماد، الرطوبة، البروتين ، إجمالي الكربوهيدرات)، كما تم تحديد إجمالي المواد العضوية، إجمالي الكربون العضوي، المحتوى العنصري الغير المرغوب فيه (الكروم والنيكل والزرنيخ والرصاص والكادميوم) والعناصر الغذائية (الصوديوم والبوتاسيوم والفوسفور والمغنيسيوم والكالسيوم والمنجنيز والحديد والكوبالت والنحاس والزنك والسيلينيوم، وعناصر أخرى (الموليبدينوم والقصدير والأنتيموني)، أميلوز، أميلوبكتين، قيمة الرقم الهيدروجيني، قدرة الربط بالماء، الكربوهيدرات الكلية القابلة للتحلل، قوة الانتفاخ والقابلية للذوبان، تم تحقيق دراسات طيفية تتضمن نمط تحويل فورييه بالأشعة تحت الحمراء وأنماط الإكس راي (حيود الأشعة السينية) للنشويات المعزولة.