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EFFECT OF EXTRACTION METHODS ON FUNCTIONAL AND PHYSICOCHEMICAL PROPERTIES OF RICE AND WHEAT STARCH

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ABSTRACT

This study was aimed to investigate the impact of starch isolation methods from wheat (Buhooth 22) and rice (Anbar) on the chemo physical , functional properties and final yield of starch .The starch isolated from wheat using three different methods (Dough ball (DB),Batter method(BM) , Alkaline method),and from rice using Alkaline extraction (NaOH 0.15%) .The obtained results showed that the yield of the isolated starch for wheat ranged from (42.6-63.0)% ,and the highest percentage achieved using alkaline isolation (NaOH 0.15%) was 61% and 63% for DBS , As for rice the highest yield was 72.30%,while the lowest yield for wheat using alkaline solution (0.50% NaOH) was (42.6)%. Amylose percentages in wheat starch reached to 55.3% in case of using 0.10% NaOH and it dropped gradually with increasing the NaOH concentration .The resistant starch percentages ranged from (0.06-0.73)% in the starch samples with significant difference among the isolation methods and also between rice and wheat samples .The results of SEM revealed that the isolation methods influenced the starch granules morphology and the chemical and functional characters as well.

Keywords: amylose, amylopectin, chemophysical characteristics, SEM analysis

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INTRODUCTION

Starch is the primary source of stored carbohydrates in various parts of plants, including seeds, fruits, tubers, and roots. Studies have confirmed that starch extracted from corn grains (Zea mays subsp. Mays L), wheat (Triticum aestivum), and rice (Oryza sativa) is the most widely consumed globally, despite the challenges associated with its purification compared to other plant sources. This is due in part to its low moisture content and the close association of starch in grains with the protein matrix (Emad and Mousa. 2022; Shanis et. al, 2019; Verma et. al, 2018). Starch is present in amyloplast cells in the form of semi-crystalline, water-insoluble granules inside the cells, arranged in alternating crystalline and amorphous layers. It consists of heterogeneous units (differing in length and molecular weight) of glucose polymers linked by α 1–4 and α1–6 bonds, forming semi-linear chains of amylose, constituting approximately 25%, with a molecular weight range of (105–106) Da. The remaining 75% comprises highly branched amylopectin with a molecular weight range of (107–109) Da. Additionally, small amounts of fats (0.1 - 1.0%) and proteins (0.05 - 0.5%) are present (Ahuja et. al, 2013). The ratio of amylose to amylopectin and their structural variation heavily depends on the plant origin of starch. The amylose to amylopectin ratio has been associated with the physical, chemical, and functional properties of starch. Starch granules containing a high proportion of amylose exhibit high levels of resilience and absorb a limited amount of water during cooking. Conversely, starch granules with a high amylopectin content (waxy starch) show high viscosity and low retro gradation (Jiawei

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et.al, 2015; Magallanes et. al, 2017). Starch is classified based on the rate of glucose release and absorption in the digestive tract into three types rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (48). RS is Known as the starch that reaches the large intestine without digestion, and chemically estimated by calculating the difference between total starch (TS), and the sum of (RDS) and (SDS), (Peter and Stintzi, 2021). The starch extraction process is primarily influenced by the solubility of proteins, the level of interactions between proteins and starch, and the properties of starch granules, including morphological features such as shape and diameter. Various methods are employed for rice starch extraction, including alkaline soaking, high-density ultrasound waves, and protease digestion. These methods exhibit different extraction efficiencies and diverse functional properties. The extraction methods for wheat starch differ from those for rice due to variations in their composition and intermolecular associations. Alkaline extraction, the batter method, and the dough ball method have been identified as the most effective in isolation and purification (Jie et. al, 2021, Shanis et. al, 2019). According to Usman *et al.* (Usman et. al, 2014), efficient protein separation can be achieved in alkaline solutions, especially when employed for rice starch isolation. This is because a significant portion of rice proteins consists of glutelns that are soluble in alkaline solutions and have high molecular weights. The advantage of this method lies in obtaining starch with high purity, along with minimal or negligible structural changes under optimal extraction conditions. However, it was mentioned that the disadvantages of alkaline extraction are that when using high concentrations, amylose leaching occurs, resulting in a decrease in starch yield. Isolating wheat starch using the dough ball method was found to be more efficient than the batter method, resulting in higher starch yield and greater purity, with lower fat and protein content. The significance of starch goes beyond being the primary source of body energy. Its high quantity, ease of isolation, and low cost, coupled with its unmatched functional properties as a thickening and stabilizing agent, and its ability to form gels, position it at the forefront of both food and industrial components. Additionally, the capability to modify its physical and chemical properties makes it suitable for specific applications in both food and industrial systems (Anneleen et. al, 2012; Le Thanh-Blicharz and Lewandowicz, 2020). Resistant starch has multiple benefits in preventing diabetes, obesity, inflammation, and promoting gut health. This is achieved through its ability to alter, diversify, enrich, and increase the abundance of beneficial bacteria in the intestines. Additionally, resistant starch serves as a source for the production of short-chain fatty acids, leading to a decrease in glucose and insulin responses, as well as fat formation (Zhang and Bao, 2021). Given the importance of wheat and rice starch as predominant elements in all dietary meals, this study aimed to determine the optimal method for starch extraction and identify the percentage of resistant starch in both local wheat and rice flours. Furthermore, the study aimed to investigate some of their functional and morphological properties.

MATERIALS AND METHODS

Wheat grains of the Buhooth 22 variety, harvested in 2021, were obtained from the Ministry of Science and Technology / Agricultural Research Department / Seed Germination Center. Anbar rice (Jasmine Anbar Rice) was purchased from local markets. The test kits for estimating resistant starch were provided by the Irish company Megazyme International Ltd.

Grinding of Grains: Rice grains grinding: It was conducted using a household coffee grinder after conditioning moisture content to 14% by adding distilled water (using the hydration equation) (Al-Muhayawy, 2018). Then grains were stored for 24 hours in a sealed plastic bottle, with stirring every two hours.

$$\textbf{Ad.Wa.ml}= \left(\frac{\textbf{100}-\textbf{R.G.M.}}{\textbf{100}-\textbf{calculated M.}}-\textbf{1}\right)\times \textbf{w.sa}$$

w.sa.=weight sample,Ad.Wa.=Add water
R.G.M.=Raw grain moisture

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The obtained powder was then passed through a sieve with a mesh size of 150µm, and stored in tightly sealed polypropylene bags at a temperature of 40 C.

Wheat Grains grinding: undertaken as described in AACC (2010) 26-31.01, using a laboratory mill (Buhler Mill 2020) with 3 breaking and 3 reduction systems at an extraction ratio of 70% (Attia, 2022). The grains moisture was raised to 15% through a conditioning process as described above. The resulting flour was passed through a 355 mesh sieve and stored as mentioned previously.

$$\textbf{Ex. R.}= \frac{\textbf{weight of resulting flour}}{\textbf{weight of resulting flour+W.B.}} \times \textbf{100}$$

Ex.R.=extraction ratio,W.B.=Weight of bran

Dough ball method extraction: Starch was extracted from wheat flour following Anneleen *et al*. (Anneleen et. al, 2012) method with some modifications. One hundred grams of wheat flour was mixed with 52 mL of distilled water in a pin-mixer to form cohesive (ideal) dough. The dough ball was washed under a weak stream of distilled water until the wash water became clear. The starchy solution was filtered through a sieve (88 microns) and then centrifuged at xg 6500 for 10 minutes. The washing process was repeated twice, discarding the supernatant each time, and the upper layer was carefully removed using a flat spoon. The starch was then dried at a temperature of 40 ± 2∘ C until a constant weight was achieved and starch chunks were ground using a coffee grinder, and the powder was passed through a 100-mesh sieve and stored in sealed containers under dry conditions. The extraction ratio was calculated using this equation:

$$\textbf{Ex.R.}= \frac{\textbf{weight of dried starch}-\textbf{W.T.F S.}}{\textbf{W.T.F S.}} \times \textbf{100}$$

Ex.R.=extraction ratio,W.T.F.S. Weight of treated flour sample

Extraction using the batter method: The method described by Anneleen *et al*. (Anneleen et. al, 2012) was followed with the suspension of 33 grams of wheat flour in 140 mL of deionized water. The batter was left for 10 minutes to ensure soaking, absorption, and swelling of the flour components. Then, it was centrifuged at xg100 for 5 min.. The batter was filtered through a sieve (88 microns) to isolate gluten protein particles, then centrifuged at xg6500 for 10 min.. The residue was washed twice, discarding the supernatant each time, and the upper layer was carefully removed using a flat spoon. The starch was dried as mentioned previously

Alkaline treatment extraction:

Extraction of wheat starch: Wheat starch was extracted from wheat flour according to the method described by Verma *et al*. (Verma et. al, 2018) with some modifications. The flour was soaked in five different concentrations of (NaOH) solution (0.10%, 0.15%, 0.20%, 0.25%, and 0.50%) at ratio of 1:6 (w/v) using magnetic stirrer for 30 minutes, then it was stored at 4±2∘C for 24 hours. Subsequently, it was centrifuged at 6500xg for 10 min, the yellowish upper layer was removed. The suspension was then re-centrifuged with the same conditions, and the supernatant was discarded each time. The upper layer was carefully removed until it disappeared. The starch was then neutralized with HCL (0.1 M) to pH (6.8-7) then re-centrifuged. The starch was dried as mentioned above.

Extraction of rice starch: Shanis *et al*. (Shanis et. al, 2019) was followed for extracting rice starch. Rice flour was soaked in a sodium hydroxide solution (0.15%) at ratio of 1:10 (w/v), for 18 hours. The remaining steps were completed as mentioned previously.

Amylose estimation: Amylose and amylopectin content were determined as described by Abeysekera *et al*. (Abeysekera et. al, 2017). The amylose content was measured using the colorimetric method of blue amylose-iodine complex. Amylopectin percentage was estimated according to the following equation (Nor Nadiha et. al, 2010):

Amylopectin %= 100 - Amylose%

Resistant starch estimation: The percentage of resistant starch was determined according to A.O.A.C method 2002.02 (AOAC, 2019.), using standardized equipment provided by Megazyme International Ireland Ltd. according to the company's instructions.

Scanning electron microscopy (SEM) examination: The samples preparation and SEM examination were done due to the

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method described in (Liu et. al, 2022). A scanning electron microscope from the Dutch company Thermos Scientific (Axia Chiemi SEM) was used.

Chemical composition: Moisture and Ash content were determined according to (A.A.C.C) (39-10.01) (08-01.01) according to (Al-Dulaimi and Nashmi, 2021, Jassim, 2020). Total fiber was determined due to A.O.A.C. method as mentioned by Al-Roumi (Al-Roumi, 2021). The protein content was determined using the Micro-Kjeldahl method, as described by Attia (Attia, 2022).

Functional properties of rice and wheat starch:

The solubility and swelling capacity: For rice and wheat starch were determined using an alkaline method (0.15% NaOH) according to (Qadir et. al, 2021).

$$\mathbf{Su.\%} = \frac{\text{D.W.with dry sample} - \text{w.of empty dish}}{Sa.W.} \times 100$$

$$\mathbf{S.Ca.\%} = \frac{\text{weight of preciptate}}{Sa.W \times (100 - \text{So.\%})} \times 100$$

So.= solubility,S.Ca.= swelling capacity,Sa.W.=------Sample weight,D.W.=dish weight

Water holding capacity (WHC) Estimation: The WHC of wheat and rice starch was calculated according to (Noraidah et. al, 2023).

$$\mathbf{WHC\%} = \frac{\text{w.of the T.with Pr.(g)} - \text{w.of theT with sa.(g)}}{\text{Sa.W.}} \times 100$$

Sa.= Sample ,W.= weight,T=tube, Pr=precipitate

Oil holding capacity (OHC) estimation: Oil holding capacity was determined according to (Anum Khan et. al, 2020) for wheat and rice starch. OHC is calculated as follows:

$$\mathbf{OHC\%} = \frac{\text{W.S.O.} - \text{original starchweight}}{\text{original starch weight}} \times 100$$

W.S.O. weight of starch with oil

Statistical analysis

The Statistical Analysis System (SAS) software (SAS, 2018) was used to analyze the data to and completely randomized design (CRD) was applied. The significant differences among the means were assigned using the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Extraction rate:

Table 1. represents the percentages of extraction rates for wheat starch which extracted by alkaline , dough ball, and batter methods, and that of rice starch. The highest yield (72.30%), for rice starch was recorded by (TR15), which was significantly different as compared to other treatments. This result is close to that was mentioned by (Wang and Wang, 2004) (71.6%). Verma *et al.* (Verma et. al, 2018.) stated that the yield of starch extracted from two different types of rice was negatively proportional to the concentration of the alkaline solution. Wheat starch yield percentages for (DBS), (T15), and (BMS) treatments were significantly higher (63, 61, and 58%, respectively), than those for (T10, T20, T25, and T 50) treatments, which were (47, 50.9, 48.3, and 42.28) %, respectively. The yield of wheat starch obtained from (DBS) (BMS) methods was similar to what was mentioned by Anneleen *et al.* (Anneleen et. al, 2012), which was (50 and 61) %, respectively for soft wheat flour. They mentioned that the yield of starch by the (DBS) method is higher due to the formation of flexible and sticky dough as a result of the gluten network formation , while in the (BMS) method, the gluten network does not develop and with the belief that washing with water only can harm the abundance of starch. It is observed from the same table gradual decrease in starch yield with increasing of alkaline solution as the highest yield of wheat starch was in treatment (T15) using (0.15% NaOH), while the lowest percentage was for (T50) with (0.50% NaOH). This is consistent with verma *et al*. (Verma et. al, 2018) findings. Wijesinghe (Wijesinghel and Gunathilakel, 2020) indicated that the yield of rice, wheat, corn, and Proso millet starches were (64.1, 54.4, 70.5, 52.8% using 0.25 NaOH%), respectively. Wang *et al*. (Wang and Copeland, 2012) explained the decrease in the yield of the extracted starch in case of using the alkaline solution attributed to the leaching of amylose from the starch granules.

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Table 1. The yield, amylose, amylopectin, and resistant starch percentages for rice and wheat starch extracted by different methods.

Starch samples	Yield%	Amylose ratio%	Amylopectin ratio%	Amylose /amylopectin ratio	Resistant starch%
Rice Starch					
TR15(0.15%alkaline)	72.30 a	d12.60	a87.40	c0.14	c0.060
CRS(commercial)	-	25.74c	74.26b	c0.34	2.520a
Wheat Starch					
DBS(dough ball method)	63.0 b	0 c21.6	78.4a b	c0.27	b0.530
BMS(batter method)	58.0 bc	0 c23.0	a b77.0	c0.29	b0.344
T10(0.10%alkaline)	47.0 c	a55.30	c44.70	a1.23	0.324 b
T15(0.15%alkaline)	61.0 b	a50.47	c49.53	ab1.01	b0.341
T20(0.20% alkaline)	50.90 c	a49.52	c50.48	ab0.98	0.336 b
T25(0.25% alkaline)	48.30 c	b45.85	c54.15	b0.84	b0.731
T50(0.50% alkaline)	c42.28	0 b44.9	c55.10	b0.81	b0.550
L.S.D.	8.724 *	6.917 *	11.229 *	*2850.	0.502 *

.(P≤0.05) *

Amylose and amylopectin content:
Table 1. shows the percentages of amylose and amylopectin values in wheat and rice starch. These values were (55.3, 50.47, 49.52, 45.85, 44.9, 21.60, 23, 12.6, 25.74) respectively. The highest percentages of amylose were recorded by the wheat flour Alkaline treatments and the amylose content dropped with the gradual increasing of the alkaline solution concentration as the lowest value noticed in the treatment (T50). There were no significant differences among (T10, T15, T20), but they were significantly different than the rest, due to the filtration of amylose during the extraction process (Anil and Harold, 2007). Wang and Copeland (Wang and Copeland, 2012) confirmed that the sites of amylose in starch granules are in the central, non-crystalline core and that amylopectin clusters are around it. During alkaline treatment of starch granules, a limited form of gelatinization occurs, leading to the formation of internal modifications, loss of amylose, and a decrease in crystallinity and the content of the double core. Wang *et al*. (Wang et. al, 2019) indicated that the amylose content in corn, wheat, and tapioca (commercial) starches was (13.47, 18.2, 15.56) %. Brij *et al*. (Brij et. al, 2020) showed that the amylose content of wheat starch extracted by the dough ball method was between 18.35 and 20.58%, which is similar to the amylose content of the DBS treatment in this study. Qadir *et al*. (Qadir et. al, 2021) indicated that the amylose content of different rice varieties ranged from 21.08 to 25.40%. This variation in amylose content is due to the differences in rice varieties, due to the plant source, as well as the environmental and agricultural conditions during the ripening period. The ratio of amylose to amylopectin plays a role in predicting the glycemic index, as high values indicate a low glycemic index (Verma et. al, 2018). The highest ratio was observed in the treatments (T10, T15, T20) with a significant difference from the rest of the treatments.

The percentage of resistant starch: Table (1) shows that commercial rice starch (CRS) recorded the highest percentage of resistant starch (2.52) , with a significant difference from the rest, followed by the wheat starch treatments T25, T50, DBS, which recorded (0.731, 0.550, 0.530) respectively .These results are lower than that found by (Noraidah et. al, 2023) for wheat flour, which was (1.08%). The lowest reading was for Anber rice starch which was lower (0.060%) than that was reported by (Arvin et. al, 2021, Woraratphoka et. al,2021).

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Chemical composition: Table 2 represents the chemical composition for rice and wheat starch. The moisture content was low for all treatments under study, reflecting the degree of drying. The moisture content ranged from 6.25 to 7.95 %, which shows a significant difference among the treatments. However, meanwhile, all percentages were within the recommended moisture level (<14%), to prevent spoilage and clumping. The ash and protein content expresses the efficiency of the extraction process for pure starch. The highest reading was (BMS, DBS) samples. As noticed being (0.24, 0.22) %, and the lowest (0.12%) was for the (CRS) sample. It has been noticed that the ash content is inversely proportional to starch yield, and the protein content for (T10 – T50) were inversely related to the concentration of alkaline extraction solution. This is because the alkaline solution disperses the protein matrix, leaving the starch free from the protein, which increases the starch recovery rate (Shanis et. al, 2019, Usman et. al, 2014). The same table shows the highest value of fibers was (1.81) % for the (DBS) treatment, with a significant difference from the rest. While the lowest value was 0.8% for (TR10), with a significant difference from the rest readings. These values were higher than the value (0.12%) reported by (Wijesinghel and Gunathilake1, 2020) in wheat and rice starch, which were extracted by 0.25% (NaOH)solution.

Table 2. Chemical composition of wheat starch extracted using different methods and rice starch extracted by alkaline solution.

ID	Treatment	Moisture %	Ash %	Protein %	Total fibers %
1	Rice starch TR15(0.15%alkaline)	7.0 ab	0.17 ab	0.39 b	0.8 c
2	CRS (Commercial)	7.30 ab	0.12 b	0.42 ab	b1.21
3	Wheat starch DBS(dough ball starch)	7.0 ab	0.22 a	0.23 C	a1.81
4	BMS(batter method starch)	6.25 b	0.24 a	0.55 a	b1.29
5	T10(0.10%alkaline)	7.50 ab	0.21 ab	0.50 ab	b1.21
6	T15(0.15%alkaline)	7.40 ab	0.19 ab	0.47 ab	29 b.1
7	T20(0.20%alkaline)	7.95 a	0.18 ab	0.43 ab	b1.36
8	T25(0.25%alkaline)	7.0 ab	0.18 ab	0.39 b	b1.27
9	T50(0.50%alkaline)	7.50 ab	0.16 ab	0.26 bc	bc1.17
	L.S.D.	1.44 *	0.096 *	0.139 *	0.388 *

.(P≤0.05) *

Swelling capacity:

Table (3) illustrates the swelling capacity for rice and wheat starch under study. All treatments achieved a significant increase in swelling capacity values with increasing of temperatures up to 95°C, at which the highest value was recorded . It is also clear that the rice starch in CRS , TR15 and the wheat starch sample T50 recorded the highest values, being (10.63, 9.89, 9.37)% respectively. Rice starch swelling capacities were lower than that reported by (40), which was (25.66) % for the Riceberrey rice variety at 90 °C . While Jasmien *et al*. (Jasmien et. al, 2016) and Azima *et al*. (Azima et. al, 2020.) reported that the swelling capacity of waxy rice starch was 33.07% and that of corn starch was 30.0% at 95 °C. This may be due to the difference in rice varieties and the difference in the ratio of amylose and amylopectin, in addition to other factors such as molecular weight distribution, chain length, as well as the degree of branching and its shape. Qadir *et al*. (Qadir et. al, 2021) stated that the presence of amylose prevents the swelling of starch granules. Therefore, the low content of amylose enhances the starch interaction with water through hydrogen bonds. As for the treatments for wheat starch, the swelling values appeared to be close for the (DBS and BMS) treatments due to their similar amylose ratios. , while the highest and lowest values were in T50 and T10

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treatments (9.37 and 8.27)%, with a significant difference from the rest, which were close to (Brij et. al, 2020) findings which ranged (9.27-13.35%)for different wheat varieties **.**

Table 3. Swelling capacity assay % for rice starch and wheat starch extracted by different methods and at different temperatures

Treatments	Swelling capacity%						
	55 ºC	60 ºC	65 ºC	75 ºC	85 ºC	95 ºC	L.S.D.
Rice starch TR15(0.15%alkaline	C1.97	b2.47C	bc4.293	B5.92	a8.79AB	10.63a A	2.98 *
CRS (Commercial)	C 3.01	ab BC 4.80	B5.20	B6.56	ab8.43AB	ab9.89A	2.77 *
Wheat starch BMS (batter method starch)	B 2.88	a B5.15	B 5.16	AB6.68	ab7.57AB	ab8.47A	2.95 *
DBS (dough ball starch)	B 2.60	a 5.11 B	B 4.92	ABb6.21	ab6.68AB	ab8.44A	2.86 *
T10(0.10%alkaline)	B 2.40	ab B4.70	B5.10	AB6.92	ab8.02A	b8.27A	2.85 *
T15(0.15%alkaline)	C1.89	a3.29 BC	B4.99	A6.01	b6.42A	ab8.39A	2.63 *
T20(0.20%alkaline)	B 3.20	B4.40ab	B5.0	AB 7.14	ab8.19A	ab8.53A	2.91 *
T25 (0.25%alkaline)	C2.10	ab B4.84	B 4.89	AB6.78	ab7.34AB	ab8.66A	2.65 *
T50(0.50%alkaline)	B 2.20	ab B4.20	B 5.08	AB6.93	ab7.80AB	ab9.37A	3.68 *
L.S.D.	2.04 NS	2.35 *	2.092 NS	2.26 NS	2.34 *	2.29 *	---
.(P≤0.050)*							

Capital letters represents the significances among the means in rows.
small letters represents the significances among the means in columns.

Solubility: Table 4 shows the percentage of solubility of alkaline-extracted rice starch and wheat starch extracted by different extraction methods at different temperatures. the percentages of solubility of the samples varied within the same treatment with the temperature increasing. The highest values were at 85°C for BMS, CRS, DBS, and TR15, respectively. Then the highest values were recorded at 95°C in the treatments T10, T15, and T50, respectively, with a significant differences. All solubility readings in this study at 95C̊ were lower than that reported by (Wijesinghel and Gunathilake1, 2020). Kaur *et al*. (Kaur et. al, 2018) found that solubility for rice starch was 5.38% at 85°C, they suggested that the increase in solubility at high temperatures due to the swelling of the starch granules until the temperature of the batter exceeds the temperature of gelatinization, which leads to the disruption of the crystalline structure of the starch granule and it opens, and then the water molecules bind to the hydroxyl groups with both amylose and amylopectin, through hydrogen bonds, the solubility increases. Table (4) also shows that the highest solubility values at 75 °C were recorded for BMS and DBS treatments, as they reached 3.73 and 2.90%, and they were lower than that mentioned by (Brij et. al, 2020) at the same temperature, for DBS method, ranging from 6.48. - 11.98% according to wheat varieties.

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Table 4. Solubility index of rice flour starch and wheat flour starch by different extraction methods and different temperatures.

Treatments	Solubility %						
	55 ºC	60 ºC	65 ºC	75 ºC	85 ºC	95 ºC	L.S.D.
Rice starch TR15(0.15%alkaline)	c0.725B	c1.03B	c1.294B	c1.85A	bc2.08A	B0.93 d	0.655 *
CRS (Commercial)	c0.99B	c1.35B	bc1.56B	cd1.55B	b2.71A	cd1.39B	0.747 *
Wheat starch BMS (batter method starch)	A3.05a	a3.07A	a3.23A	a3.73A	a3.66A	cd1.50B	0.902 *
DBS(dough ball starch)	bc1.47B	a b2.61A	a2.65A	b2.90A	b2.70A	bc2.60A	0.712 *
T10(0.10%alkaline)	bc1.33C	a2.79B	a2.87B	bc2.67B	c1.07C	a4.27A	1.03 *
T15(0.15%alkaline)	c0.754B	bc1.45AB	c1.08AB	d1.03AB	c1.53AB	b3.05A	2.15 *
T20(0.20%alkaline)	b c1.63B	bc1.65B	bc1.75B	bc2.39A	bc2.13A	cd1.59B	0.605 *
T25 (0.25%alkaline)	b1.81	b2.07	b2.15	c2.03	bc2.21	c2.01	0.457 NS
T50(0.50%alkaline)	bc1.39B	bc1.55B	bc1.79A	c1.73A	c1.77A	bc2.40A	0.691 *
L.S.D.	0.702 *	0.694 *	0.651 *	0.759 *	0.704 *	0.821 *	---

.(P≤0.05) *

Capital letters represents the significances among the means in rows.
small letters represents the significances among the means in columns.

Water binding capacity (WBC):

Table 5 shows water binding capacity and pH of rice and wheat starch samples. The percentages ranged from (85.04 - 109.30) % with a significant difference. The WBC of rice starch samples (TR15 and CRS) were (90.90 and 103.69) %, which are lower than what was mentioned by (Qadir et. al, 2021), which ranged from (183.33 - 191.41) %. This difference in the percentages of WBC may be due to the difference in rice varieties, which is represented by the structural differences inside the starch granules, which provide opportunities for associations between starch chains through different bonding forces, thus exposing hydrophilic areas to the external environment. The increase in WBC percentages was attributed to the weak interactions of starch chains, which enhance the interaction of water with polymers through hydrogen bonds. As for wheat starch, the treatments (BMS, DBS, and T50) recorded the highest percentages of WBC and differed significantly from the rest treatments, and were (109.3, 105.62, and 99.64) %. Wang *et al*. (Wang et. al,2019) found a similar percentage for wheat starch, being (97.12 ± 2.16) %. Bashir *et al*. (Bashir et. al, 2016) stated that the percentage of WBC was (0.24 ± 85) %, which is almost identical to the values in the current study and for the same extraction method for the treatments (T25 - T10), which were (85.04-86.82) % as shown in Table (5).

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Table 5. Oil binding capacity, water binding capacity, and pH values for rice starch and wheat starch extracted by different methods

ID	Treatment	Oil binding %	Water binding %	pH
1	Rice starch TR15(0.15%alkaline)	b107.39	90.90 a	ab6.50
2	CRS (Commercial)	134.10a	103.69 a	6.90ab
3	Wheat starch BMS (batter method starch)	b c104.87	109.30 a	5.20b
4	DBS (dough ball starch)	88.20 bc	105.62 a	5.50b
5	T10(0.10%alkaline)	99.50 bc	b85.04	6.50a
6	T15(0.15%alkaline)	c82.06	b85.20	6.70ab
7	T20(0.20%alkaline)	c73.87	b85.36	6.60ab
8	T25 (0.25%alkaline)	c75.36	b86.82	6.80ab
9	T50(0.50%alkaline)	c84.33	a99.64	7.0a
	L.S.D.	22.371 *	19.667 *	1.433*

.(P≤0.05) *

Oil binding capacity (OBC)

Table (5) shows the OBC% for rice and wheat starches. The highest percentages of OBC were for the rice starch treatments (TR15, CRS) and wheat treatment (BMS) (107.39, 134.10, 104.87 %) respectively. OBC value for commercial rice starch was significantly higher than that for alkaline-extracted rice starch. This finding was also higher than that mentioned by Odi and Sinija (Odi and Sinija, 2021), which was 90%, and they assured that starch with good OBC can be used to enhance the taste sensation in addition to its use as a fat holding agent. As for the treatments for wheat starch, they ranged from (104.87-73.87) %. The variation in OBC is due to the difference in extraction methods, and its effect on changing structures and display hydrophobic groups. The OBC% for the DBS treatment reached to (88.20) %, which is lower than what was mentioned by (Brij et. al, 2020) for the same treatment, which ranged from (96.0-117.0) % with different wheat varieties. The variation of the percentage in OBC was due to the alkaline treatment that may leads to a change in the morphology of the starch granule, forming a rough surface and cavities that increase the lipophilic properties of the starch (Marta et. al, 2022).

Electron microscopy: Figure 1(a-i) shows the microscopic structure of wheat and rice starch granules under study, which included the microscopic structure of wheat starch isolated by alkaline extraction using different concentrations of (NaOH) solutions, Figures (a-e). Figures (f and g) represent wheat starch granules (DBS and BMS) . Figure (h) represents rice starch granules alkaline-extracted (0.15 % NaOH), while Figure (i) represents commercial rice starch granules .The wheat starch granules are having a bimodal size distribution, characterized by the presence of large lenticular (A-type) granules and small round (B-type) granules. The size of wheat starch granules appearing in Figure (a) ranges from approximately 3.5 to 25 micrometers µm. Jasmein *et al*. (Jasmien et. al, 2016) stated that the size of type (A) granules ranged from 20-32 µm and type (B) granules ranged from 2-10 µm. As for rice starch granules, which are shown in Figure (h), they appear as small, multi-faceted granules with an average diameter of 2.2-7.2 µm. This is in agreement with De Souza *et al*. (De Souza et. al, 2016) results who stated that the diameter of rice starch granules was about 3-8 µm . Verma *et al*. (Verma et. al, 2018) mentioned that the size of the particles is inversely proportional to the WBC%, hence in this study the rice starch WBC% samples was higher than that of wheat starch T10 – T25 this is because the rice starch granules where smaller ,the granule less than 10 mµ shows greater WBC% which in tearn gives stronger dough with less elasticity .It is obvious from Figure (a), that the starch granules are still sticked to

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each other by protein molecules and retain their shapes , and appears in spherical to lenticular form. Figures (b , c) shows that the granules are connected to each other to a lesser extent, and there are more single granules. Some of them still retain their shapes, while others have begun to elongate. Figures (d , e) show a jelly-like appearance and loss of granular formation due to alkaline gelatinization. . The results of this study were consistent with Noraidah *et al*. findings (Noraidah et. al, 2023). The decrease in amylose content is a phenomenon that accompanies alkaline treatment and is attributed to disruption of the amorphous regions that contain amylose chains . It is possible that the ions in the alkaline solution diffuse into the amorphous regions which is rich in amylose, and thus the breaking of the internal bonds between the molecules causes swelling of the granules to a high degree with the exudation of amylose, and thus a decrease in the amylose content associated with the alkaline treatment. Figure (h) shows rice starch granules after treatment with an extraction solution of NaOH 0.15% (Usman et. al, 2014). The granules are multi-faceted with sharp angles. The surfaces of the granules are smooth and flat, and some have a slight depression, with no pores observed on the surface. There is no obvious damage or structural defects or pores on the surfaces. Figure(i), which represents commercial rice starch, shows the granules are intact, oval to lenticular in shape, and are stuck together in clusters. The granules appear in different diameter ranging from 3.5-25 mμ. Nadiha *et al*. (Noraidah et. al, 2023) indicated that the extent of damage to starch granules from plant sources in alkaline treatment varies, and the appearance of some cavities and distortions on the surface of the starch granules in the SEM test does not mean that others are not, as they may not be noticed in the Figures ,but they are large enough to allow through the water, enzymes, or reagents. In general, it was observed that the alkaline treatments at concentrations of 0.10 - 0.20 % did not show distortion in the granular shape, and no clear defects were observed on the surface of the granules. However, concentrations above 0.24 % showed a change in the granular organization of starch. This is in agreement with the sources (De Souza et. al, 2016; Jinwen et. al, 2014; Shanis et. al, 2019), which confirmed that swelling of the granules and a major disruption of the granular formation of extracted starch occurs when using alkaline solutions above 0.24 %. The hydrogen bonds of amylopectin can be broken, which leads to a change in the structure of the granules. When these bonds are broken by alkaline treatment, the granules gradually break apart and appear to be fused together. They also explained that when alkaline gelatinization occurs, NaOH can react with the lipids that are naturally present in amylose after penetrating the starch granule to form sodium salts of fatty acids. These fatty acids are surface-active agents and form stable complexes with amylose. Figures (f , g) represent starch granules extracted by the DBS and BMS methods, respectively. It is observed that wheat starch granules in both treatments appear in lentil-shaped to oval shapes with a clear depression in some of them. Their sizes were less than 35 mμ, which is close to what was mentioned by Anneleen *et al*. (Anneleen et. al, 2012) where the size of the granules for the same treatments was less than 40 mμ. Small protein molecules are also visible, which are still attached to the starch granules. It is observed that their presence is higher in Figure(g) BMS than in DBS, which forms a viscous and elastic dough whose properties depend on the formation of the gluten network. On the other hand, in the BMS method, a small amount of the gluten network is formed, if any, in addition to the difference in the dilution ratio between the two methods (added water). Therefore, these differences will play a role in the difference in the proportions of the remaining components associated with the starch granul.

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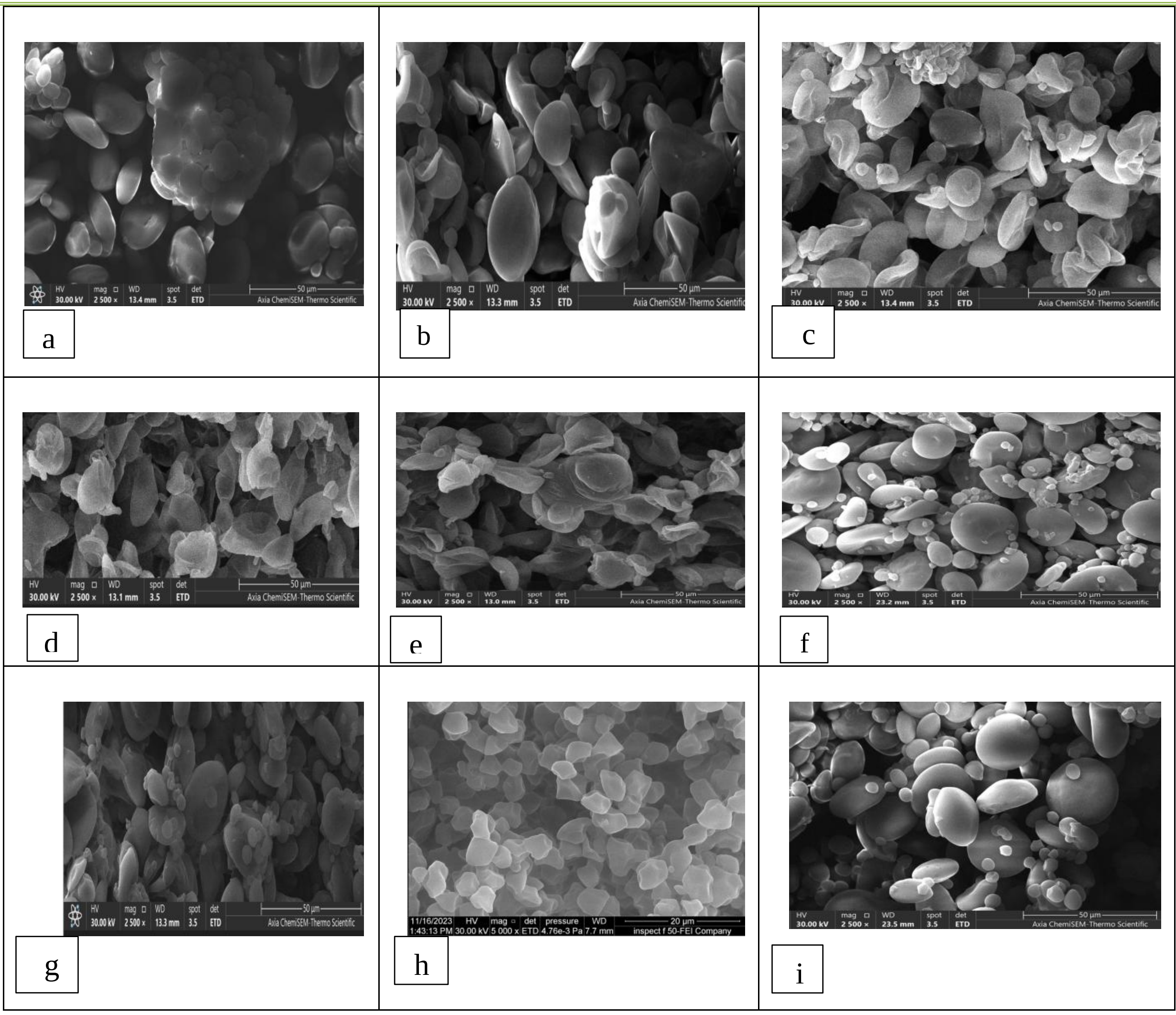

Figure 1. The scanning electron micrographs of starches isolated from wheat and rice at (2500x) magnifications.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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تأثير طرائق الاستخلاص على الخصائص الوظيفية والكيموفيزيائية للنشا المستخلص من رز العنبروقمح بحوث 22

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المستخلص

هدفت الدراسة الحالية الى تحري تأثير طرق استخلاص النشا من القمح صنف (بحوث 22) والرز (العنبر) على الخصائص الكيموفيزيائية والوظيفية والحصيلة النهائية للنشا.أستخدمت ثلاث طرق لعزل نشا القمح وهي (كرة العجين، طريقة الملاط، الاستخلاص القلوي) ولعزل نشا الرز أستخدم الأستخلاص بالمحلول القلوي (NaOH 0.15%). أظهرت النتائج ان حصيلة النشا المعزول من دقيق القمح كانت تتراوح بين (42.6 –63.0)% وأن اعلى حصيلة تحققت بأستخدام محلول القلوي (NaOH) بتركيز 0.15%وسجلت 61% و63.0% لطريقة (DBS)، أما للرز فكانت أعلى حصيلة 72.30%، بينما لوحظت أقل حصيلة لنشا القمح وسجلت 42.6% بأستخدام محلول القلوي بتركيز (0.50)% . النسبة المئوية للأميلوز في نشا القمح حققت 55.3% عند أستخدام المحلول القلوي بتركيز 0.10% وانخفضت تدريجياً مع ارتفاع تركيز محلول الأستخلاص . النسبة المئوية للنشا المقاوم تراوحت بين (0.060–0.73)% في عينات النشا بوجود فارق معنوي مابين المعاملات الخاصة بنشا القمح وما بين عينات محصولي الرز والقمح . نتائج فحص الـ (SEM) أظهرت تأثير طرق الأستخلاص على مورفولوجية حبيبات النشا والخصائص الكيمياوية والوظيفية .

كلمات مفتاحية:الاميلوز،الاميلوبكتين،خصائص كيموفيزيائية،مسح المجهر الالكتروني.