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Comparing honey quality by estimating the activity of diastase enzyme for honey samples in the Saudi markets

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Abstract:

A honey's quality is determined mostly by the activity of its enzymes. The objective of this work is to compare the quality of seven kinds of honey from three countries by measuring the activity of diastase enzyme activity number using UV / VIS Spectroscopy devices. All types are found all over the Kingdom of Saudi Arabia markets. These types were represented by Sidr honey; Saudi Sidr honey (wild and south), Yemen Sidr honey Kashmiri Sidr honey, and Talh honey including Saudi Talh and Yemen Talh honey. Black acacia honey was included Saudi type only. The obtained result revealed a remarkably significant increase in the average value of diastase number (DN) activity (estimated by using UV / VIS Spectroscopy devices) in Saudi Talh honey (STH) and Saudi Sidr wild honey (SSWH) were 70.66, 60.13 respectively. However, the average DN activity in Saudi Sidr South honey (SSSH) had approximately the half number of SSWH (32.16), while the DN activity of Saudi black acacia (SBAH) and Yemen Talh honey (YTH) was approximately the same (26.74,24,53) respectively. The lowest average value of DN activity was recorded in Yemen Sidr honey (YSH) and Kashmiri Sidr honey (17.17, 17.4) respectively. In conclusion, STH and SSWH have the greatest DN activity which reflects their potential vital role among different kinds of honey in K.S.A. The DN activity is necessary to enhance the nutritional the honey.

Keywords: UV/visible spectroscopy; honey; diastase activity, sidr, Talh, Black acacia

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INTRODUCTION

Honey is a naturally occurring sweetener with beneficial qualities (antioxidant, antibacterial, and antiparasitic) (**Osés et al., 2016; Sancho et al., 2016**) as well as potential benefits (anti-inflammatory, anti-hypertension, prebiotic, and probiotic effects) (**Machado De-Melo et al., 2018).** During the last decades, honey consumption has increased in Saudi Arabia because it is a natural product composed of sugars, enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, and aromatic substances **(Viuda-Martos et al., 2008; Da Silva et al., 2016**). The composition, color, aroma, and flavor of honey depend mainly on the flowers and

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geographical regions involved in its production, and are affected by processing, manipulation, packaging, and storage time **(Guler et al., 2007; Insuasty-Santacruz et al., 2017).**

Most of the enzymes in honey are diastase, invertase (a-glucosidase), glucose-oxidase, catalase, and acid phosphatase, all of which are present in low concentrations (**Sak-Bosnar et al., 2012**)**.** In addition to enhancing the nutritional and medicinal properties of honey, diastase (α - and β -amylase) is one of the most significant enzymes and is used as a key indicator when assessing the quality of honey (**Kedzierska-Matysek et al., 2016**). The origin of this enzyme in honey has been attributed to the salivary secretions of bees, or its presence in pollen, or nectar. The most widely recognized hypothesis claims that diastase in honey was first discovered in the saliva of bees while they were collecting nectar from blossoms (**Bonvehi et al., 2000**). The diastase activity is usually expressed in Schade units, often referred to as the diastase number (DN), which is defined as the quantity of enzyme that will convert 0.01 g of starch to the specified endpoint in 1 h at 40 °C under the test circumstances. Following the Honey Quality and International Regulatory Standards, every retail honey must have a diastase activity of at least 8, determined after processing and blending, and honey with naturally low enzyme contents must have an activity of at least 3. The standard wet chemical approach for DN determination is laborious and complicated, and it does not satisfy the need for real-time and quick monitoring of enzyme activity during the concentration's heat treatment of honey **(Kedzierska-Matysek et al., 2016)**. A quick and non-destructive way to measure DN is urgently needed.

Accordingly, this work aims to evaluate the diastase activity number in different honey samples from Saudi markets.

MATERIALS AND METHODS

1. Honey samples

Three types of honey (seven according to the country of origin) were collected from KSA markets (Sidr, Talh, and Black acacia), and stored in a container at 4°C until analysis. According to the country of origin, the estimated honey included seven kinds as follows:

i. Four kinds of Sidr honey from Saudi Arabia (wild and south), Yemen and Kashmiri
ii. Two Talh honey from Saudi and Yemeni
iii. Black acacia honey from Saudi Arabia.

A total of 70 samples of honey (10 samples for each kind) were collected to estimate the activity of diastase.

2. Chemicals and reagents

Iodine stock solution, iodine solution (0.0007 N), acetate buffer - pH 5.3, sodium chloride solution (0.5 M), starch solution 2%.

3. Instrumentation and experimental conditions

Spectrophotometer reader at 660 nm and Water bath at 40+0.2°C. The absorbance of the solutions at 660 nm was determined using a HACH LANGE DR/3900 spectrophotometer. The test was performed under normal conditions (25°C, normal humidity), and the solution was warmed at 40 C.

4. Analytical procedures

i. Preparation of test sample

10.0 g honey is weighed into a 50 - ml beaker and 5.0 ml acetate buffer solution is added, together with 20 ml water to dissolve the sample. The sample is completely dissolved by stirring the cold solution. 3.0 ml sodium chloride solution was added to a 50 ml volumetric flask and the dissolved honey sample was transferred to this and the volume adjusted to 50 ml. Also, the honey was buffered before encountering sodium chloride.

The starch solution is warmed to 40°C and 5 ml pipetted into 10 ml of water at the same temperature and mixed well. I ml of this solution is pipetted into 10 ml 0.0007 N iodine solution, diluted with 35 ml of water, and mixed well. The color is read at 660 nm against a water blank using a 1 cm cell.

The absorbance should be 0.760+0.020. The volume of added water is adjusted to obtain the correct absorbance.

ii. Absorbance determination

10 ml honey solution was added to a 50 ml graduated cylinder and placed in a 40°C +0.20°C water bath with a flask containing starch solution. After 15 min, 5 ml starch solution was added to the honey solution, mixed, and started stop-watch. At 5 min intervals remove 1 ml aliquots and 10 ml 0.0007 N iodine solution was added followed by mixing and dilution to 35 ml. The absorbance was recorded at 660 nm in aliquots at intervals until an absorbance of less than 0.235 is reached.

iii. Expression of results

The absorbance is plotted against time (min) on a rectilinear paper. A straight line is drawn through at least the last three points on the graph to determine the time when the reaction mixture reaches an absorbance of 0.235. Divide 300 by the time in minutes to obtain the diastase number (DN). This number expresses the diastase activity as ml 1 percent starch solution hydrolyzed by the enzyme in 1 g of honey in 1 h at 40°C. This diastase number corresponds with the Gothe-scale number. Diastase activity = DN = ml starch solution (1 percent) / g honey/h at 40°C. (SASO 102/1990).

RESULTS

For all types of honey, ten samples from each type were investigated to estimate the diastase number (DN) activity three times at regular intervals.

As shown in Table (1), the average diastase number (DN) for ten samples (3 replicates for each sample) from Saudi Talh honey (STH) appeared in the highest range (70.66) with reference range to World Health Organization (WHO). The frequency of DN for ten samples appeared with a standard deviation (SD) equal to 6.75 (Figure 1).

The investigated samples from Saudi Sidr wild honey (SSWH) revealed DN activity (60.13) less than the DN STH (Table 2). The frequency among the ten samples of SSWH showed a mean SD equal to 8.5(Figure 2).

As shown in Table (3), the average diastase number (DN) for ten samples (3 replicates for each sample) from Saudi Sidr South honey (SSSH) appeared in a lower value than SSWH (32.16). The frequency of DN for ten samples from SSSH appeared with an SD equal to 5.87 (Figure 3).

The investigated samples from Saudi black Acacia honey (SBAH) revealed DN activity (26.74) less than the DN SSSH (Table 4). The frequency among the ten samples of SBAH showed a mean SD equal to 4.43(Figure 4).

In Yemen Talh honey (YTH), the recorded value of diastase number activity of ten samples appeared approximately near to the value noticed in SBAH (24.53) (Table 5) The frequency of DN for YTH samples appeared with SD 5.96 (Figure 5)

The mean value of diastase number activity in Yemen Sidr honey (YSH) and Kashmiri Sidr honey (KSH) samples appeared approximately the same (17.17 and 17.4) respectively (tables 6&7) while the SD frequency differed (2.1 and 3.67) among the samples respectively (Figures6&7).

In a comparative account, the average of DN in STH samples is the highest one (70. 66) followed by Saudi Sidr wild honey (SSWH) (60.13) while in SSTH honey the activity of DN was approximately half that of SSWH (32.16). Comparatively, the average DN in SBAH and YTH was approximately the same (26.74,24.53) respectively. On the other hand, YSH and KH have approximately revealed the same average value of DN (17.17, 17.4) respectively (Table 8).

Table.1: The diastase number activity and their mean (3 replicate) in Saudi Talh honey

(DN) Sample	DN1	DN2	DN3	Average of DN	SD
1	85.7	66.6	90.1	80.80	12.49
2	62.5	60	66.6	63.03	3.33
3	60	66.6	75	67.20	7.52
4	60	69.7	66.6	65.43	4.95
5	75	60	75	70.00	8.66
6	75	76.9	66.6	72.83	5.48
7	78.9	69.7	75	74.53	4.62
8	85.7	75	85.7	82.13	6.18
9	62.5	66.6	69.7	66.27	3.61
10	66.6	60	66.6	64.40	3.81
				70.66	**6.75**

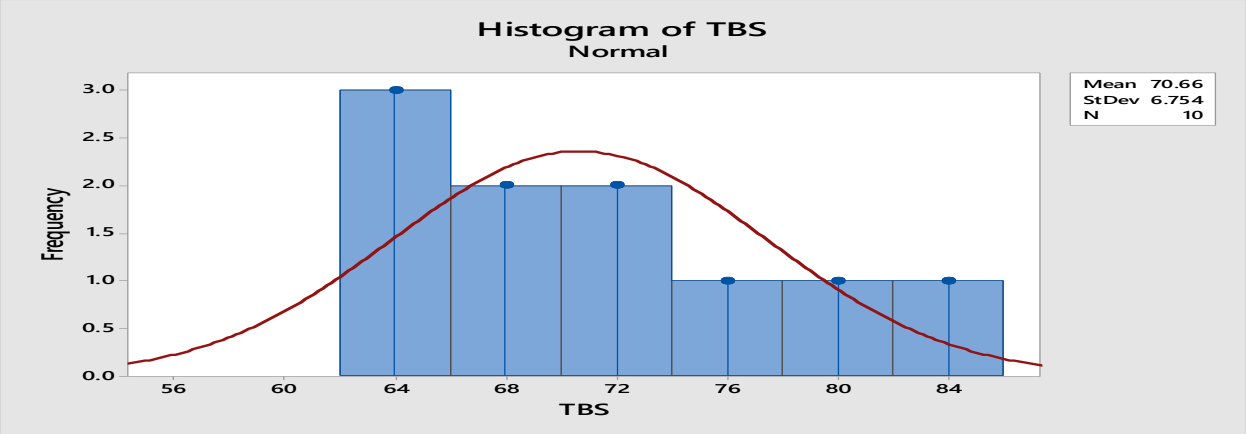

Fig.1. Histogram illustrating the mean value of DN activity in Saudi Talh honey (STH)(70.66)

Table.2: The diastase number activity and their mean (3 replicate) in Saudi Sidr honey (Wild honey)

(DN) Sample	DN1	DN2	DN3	Average of DN	SD
1	66.60	62.50	68.20	65.77	2.94
2	60.00	66.60	62.50	63.03	3.33
3	66.60	60.00	61.20	62.60	3.52
4	40.00	40.00	37.50	39.17	1.44
5	63.80	60.00	66.60	63.47	3.31
6	60.00	66.60	60.00	62.20	3.81
7	65.20	60.00	66.60	63.93	3.48
8	66.60	60.00	66.60	64.40	3.81
9	62.50	60.00	75.00	65.83	8.04
10	50.00	60.00	42.80	50.93	8.64
				60.13	**8.50**

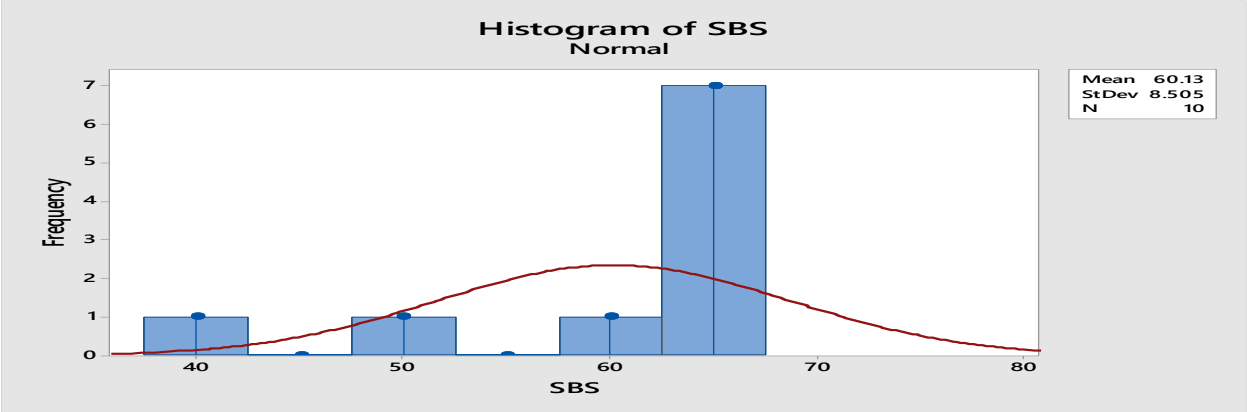

Fig.2. Histogram illustrating the mean value of DN activity in Saudi Sidr wild honey (SSWH) (60.13)

Table.3: The diastase number activity and their mean (3 replicate) in Saudi Sidr South honey

(DN) Sample	DN1	DN2	DN3	Average of DN	SD
1	31.5	37.9	33.3	34.23	3.30
2	33.3	33.3	31.5	32.70	1.04
3	32	30	31.5	31.17	1.04
4	37.5	33.3	34.1	34.97	2.23
5	24	23.4	25.4	24.27	1.03
6	38.4	37.5	35.3	37.07	1.59
7	24	25	23.4	24.13	0.81
8	37.5	35.3	40	37.60	2.35
9	25	25	25.4	25.13	0.23
10	40.5	40.5	40	40.33	0.29
				32.16	**5.87**

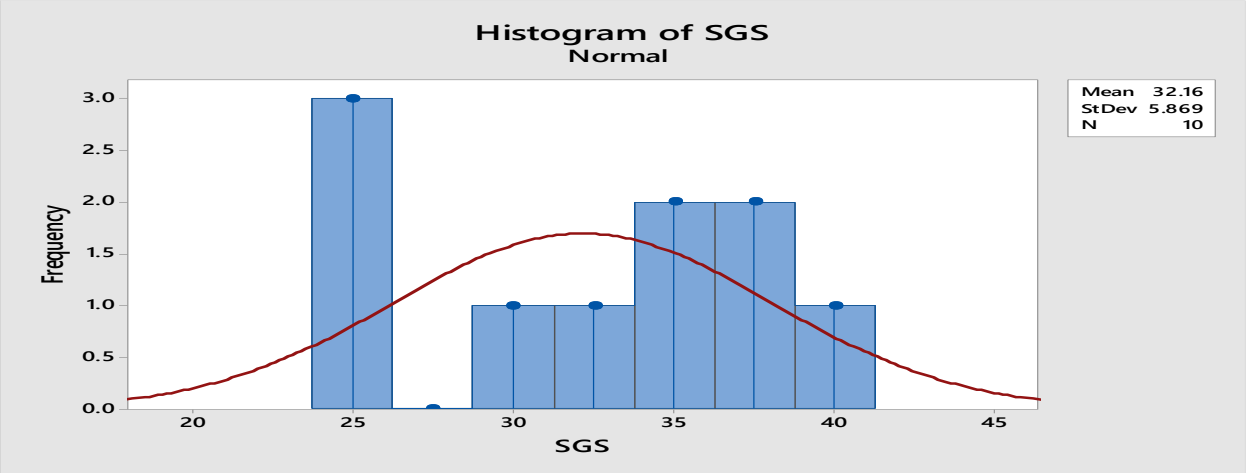

Fig.3. Histogram illustrating the mean value of DN activity in Saudi Sidr South honey (SSSH) (South honey) (32.16).

Table.4: The diastase number activity and their mean (3 replicate) in Saudi black acacia honey

(DN) Sample	DN1	DN2	DN3	Average of DN	SD
1	33.3	30	33.5	32.27	1.97
2	30	31.5	27.3	29.60	2.13
3	22.2	30	20	24.07	5.25
4	31.5	30	22.2	27.90	4.99
5	30.6	25	30	28.53	3.07
6	26.3	25	30	27.10	2.59
7	17.2	20	20	19.07	1.62
8	30	20	31.5	27.17	6.25
9	21.4	21.4	17.6	20.13	2.19
10	31.5	33.3	30	31.60	1.65
				26.74	**4..43**

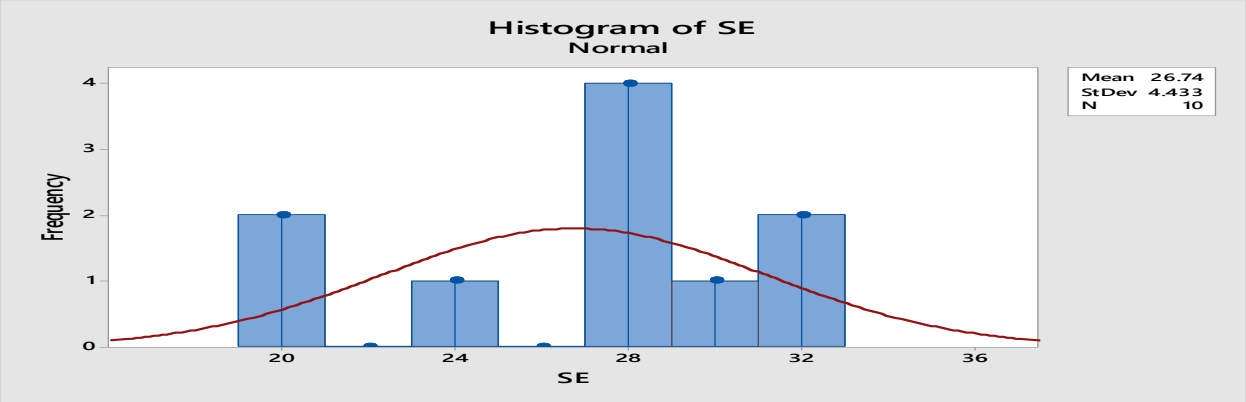

Fig.4: Histogram illustrating the mean value of DN activity in Saudi black acacia honey (SBAH) (26.74)

Table.5: The diastase number activity and their mean (3 replicate) in Yemen Talh honey

(DN) Sample	DN1	DN2	DN3	Average of DN	SD
1	20.6	20	21.4	20.67	0.70
2	30	30.6	30	30.20	0.35
3	20.3	20	22.2	20.83	1.19
4	30	30	28.5	29.50	0.87
5	20	21.4	20.6	20.67	0.70
6	31.5	30	31.5	31.00	0.87
7	20.6	20.6	20	20.40	0.35
8	34.1	30	38.4	34.17	4.20
9	20.9	20	20.9	20.60	0.52
10	15	18.2	18.7	17.30	2.01
				24.53	**5.96**

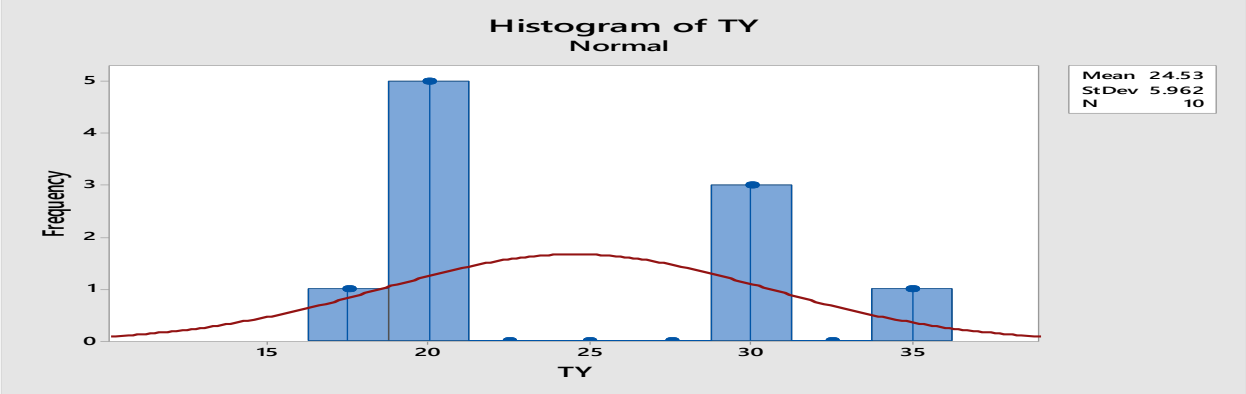

Fig.5: Histogram illustrating the mean value of DN activity in Yemen Talh honey (YTH) (24.53)

Table.6: The diastase number activity and their mean (3 replicate) in Yemen Sidr honey

(DN) Sample	DN1	DN2	DN3	Average of DN	SD
1	15.9	15.1	16.6	15.87	0.75
2	15.2	15.9	15	15.37	0.47
3	17.2	15.7	21.4	18.10	2.95
4	15.9	15.7	20	17.20	2.43
5	18.7	20	19.3	19.33	0.65
6	16.8	16.4	20	17.73	1.97
7	13.1	12	18.2	14.43	3.31
8	13.3	14.6	15	14.30	0.89
9	20	20	20.6	20.20	0.35
10	17.6	20	20	19.20	1.39
				17.17	**2.10**

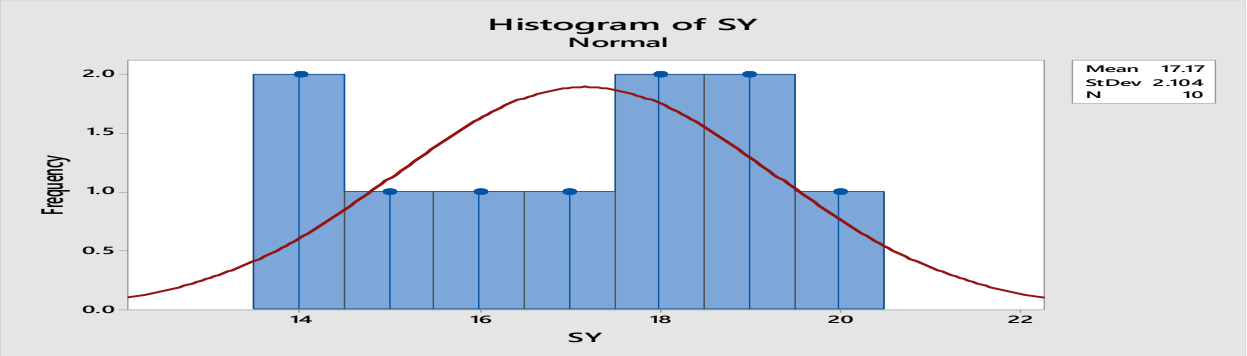

Fig.6: Histogram illustrating the mean value of DN activity in Yemen Sidr honey (YSH)(17.17)

Table.7: The diastase number activity and their mean (3 replicate) in Kashmiri Sidr honey

(DN) Sample	DN1	DN2	DN3	Average of DN	SD
1	12.8	13.3	12	12.70	0.66
2	20.9	20.6	25	22.17	2.46
3	17.6	20	20	19.20	1.39
4	15.3	15	15	15.10	0.17
5	13.9	14.1	12	13.33	1.16
6	15	12.7	17.6	15.10	2.45
7	23.1	25	23.1	23.73	1.10
8	18.7	17.1	20	18.60	1.45
9	20	15.5	20	18.50	2.60
10	15.5	16.2	15	15.57	0.60
				17.4	**3..67**

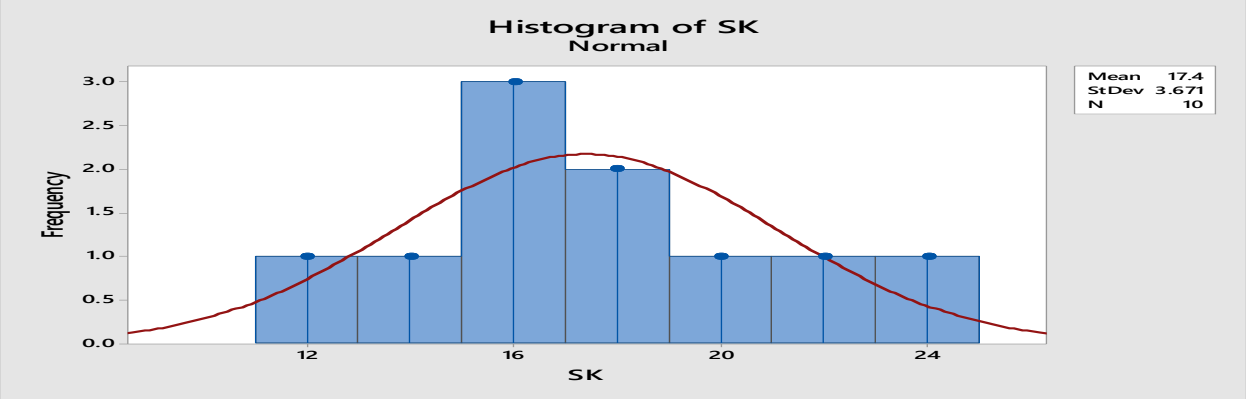

Fig.7: Histogram illustrating the mean value of DN activity in Kashmiri Sidr honey (KSH) (17.4)

Table 8: Showing a comparative account of the mean diastase number activity among the different types of studied honey.

Type	STH	SSWH	SSSH	SBAH	YTH	YSH	KSH
Mean	**70.66**	**60.13**	**32.16**	**26.74**	**24.53**	**17.17**	**17.4**

DISCUSSION

Many kinds of honey are consumed in Saudi Arabia, either domestically produced by beekeepers or imported from other nations. However, Sidr honey and Talh honey are the most widely used varieties of locally produced honey. Both are unifloral honey; the former is made from *Ziziphus spina-christi L.* floral nectar, while the latter is made from *Acacia gerrardii Benth* (**Shafin et al., 2014; Bobiş et al., 2021).** Accordingly, this study aimed to evaluate the quality of Saudi Sidr (south and wild), and Talh honey in comparison with the other five kinds of honey that differ in natural flower nectar and geographical distribution through estimation of diastase number activity.

The results of the present study revealed a significant elevation in the mean value of diastase number activity in the Saudi Talh honey (70.66), followed by Saudi Sidr wild honey (60.13) while Saudi Sidr south honey appeared significantly lower than them (32.16) but higher than Saudi acacia honey, Yemen honey (Sidr and Talh) and Kahmiri honey (26.74, 24.53,17.7 and 17.4) respectively. Previous reports declared that the flower nectar from Talh and Sidr in different regions of Saudia Arabia is rich with several antioxidants and various bioactive compounds like vitamins, phenolics, flavonoids, and fatty acids. The vitamins identified so far are vitamins A, C, and E. These compounds have been proven in the biosynthesis of honey enzymes, especially diastase **(Muhammad et al., 2015). Owayss et al**. (2019) found that honey originating from Sidr (*Ziziphus spina-christi* L.) and Talh (*Acacia gerrardii* Benth.) trees in Saudi Arabia exhibited substantial antimicrobial activity against pathogenic gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*), gram-negative bacteria (*Escherichia coli*, *Salmonella enteritidis*), and a dermatophytic fungus (*Trichophyton mentagrophytes*).

The bioactive phytochemical content of Saudi Sidr flowers has been linked in several studies to interesting antioxidant, anti-inflammatory, and anticancer activities. Polyphenols and flavonoids are the principal antioxidant bioactive compounds that have the potential to serve as free radical scavengers **(Zerrouk et al., 2018).** Polyphenolic profiles, such as carvacrol and its isomer thymol, are found in *Ziziphus* Spina-Christi honey **(Mărgăoan et al., 2012).** Both include compounds that may induce cytotoxicity, cell cycle arrest, apoptosis, and antimetastatic activity, and may lead to inhibition of several cellular damage pathways (**Sampaio et al., 2021).**

Previous studies on these two varieties of honey (Talh and Sidr) mostly examined their antibacterial activity, and generally speaking, there aren't many studies on the phenolics composition and other phytoconstituents in honey samples from Saudi Arabia (**Al-Nahari et al., 2015; Owayss et al., 2020)**. **In conclusion**: Talh and Sidr (especially wild type) Saudi honey has a higher diastase activity number comparatively with Yemen (Sidr and Talh) and Kashmiri honey. This is fundamentally attributed to the vital antioxidant constituents (flavonoids and polyphenolic compounds) in the Talh and Sidr flower nectar.

Conflict of interest

All authors declared that there were no conflicts of interest.

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