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Effect of Storage Time on Physiochemical and Microbial Properties of Saffron

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ABSTRACT

In the present study an investigation was done on physiochemical and microbial properties of saffron during 12 months after harvesting. Saffron was harvested from Torbat Heidarieh (a city in Khorasan Province), stigmas were separated by workers and the samples were dried in sieve by electrical heater (Spanish method) at 55^0C for 45 minutes until they reached to the desired moisture content (according to Saffron National Standard). Second sorting was done, and then the samples were packed in polyethylene films at 20-25 ^{0}C and stored in lab. Sampling were taken monthly and analyzed for physiochemical and microbial properties including: moisture content, aqueous extract, crocin, picrocrocin, saffranal, total count, coliform and mold count. Moisture content, aqueous extract, crocin, picrocrocin and total count decreased during storage; however saffranal showed an increasing trend. Analysis of data indicated that the largest variations during one year storage has been occurred from the beginning of harvesting time (December) to 8 months after storage (July), and after that variations were not statistically different (P>0.05).
Key word: Saffron; storage; crocin; Saffranal; total count.

1. INTRODUCTION

Saffron with its unique aroma, color, and flavor can by no means be considered a new introduction to 21st century cuisine and medicine. In fact, the history of saffron usage dates back nearly 3000 years, spanning many continents, civilizations, and cultures [6].

Saffron's name is derived from the Arab word for yellow, a name reflecting the high concentration of carotenoid pigments present in the saffron flowers' stigmas which contribute most to the color profile of this spice.

Saffron, the highly desirable golden spice, is the dried elongated stigmas and styles of the blue-purple saffron flower (Crocus sativus, L.), a member of the Iridaceae (iris) family with origins in the Middle East. At nearly 40–50 $ per gram, it is the world's most expensive spice.

It is estimated that it takes approximately 75,000 crocus blossoms or an astounding 225,000 stigmas to produce just one pound of this unique spice. The stigmas must be hand-picked from the delicate blossoms upon opening to preserve the desirable volatile components before evaporating in the heat of the day [10].

With its unmatched signature bitter-like taste, slightly metallic sub-notes, and pungent hay-like aroma, saffron has found many precious uses ranging from fragrances to dyes to medicines, but it is especially favored as both a flavoring and coloring agent in food. While saffron is more tolerant to increasing temperatures, it easily degrades in the presence of light and oxidizing agents. As a result, the best saffron is usually sold whole (not powdered) in air-tight containers absent from light sources so as to preserve its integrity.

The limited production of saffron and its extremely high price have unfortunately made it the object of frequent adulteration at least as far back as the Middle Ages.

Today, a series of analytical methodologies have been developed to determine not only the quality but also to determine the adulteration type and level in the saffron. Natural and artificial additives such as coloring agents (e.g. ground paprika/turmeric common in ground saffron, water/moisture, and glycerin in intact saffron), organic compounds (e.g. honey and oil), and inorganic compounds (borates, sulfates, chlorides, and carbonates) have been used throughout the centuries [9].

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Therefore, to ensure saffron's authenticity and quality, saffron is certified in the international trade market following the International Organization for Standardization (ISO) 3632 Normative since 1993 [14].

Currently, the most popular usage of saffron continues to be as a food coloring and flavoring agent but there also appears to be adequate evidence to support the therapeutic benefits related to saffron consumption and also for its use in mediating various health disorders. These desirable benefits have been ascribed to the various chemical components found in varying amounts within the stigma themselves.

Chemical composition analyses have revealed a saffron composition of approximately 10% moisture, 12% protein, 5% fat, 5% minerals, 5% crude fiber, and 63% sugars including starch, reducing sugars, pentosans, gums, pectin, and dextrins (% w/w). Trace amounts of riboflavin and thiamine vitamins have also been identified in saffron [20]. Ranges of all chemical constituents can vary greatly due to growing conditions and country of origin.

Various analytical studies have been conducted to characterize the large number of potential biologically active compounds found within saffron. The four major bioactive compounds in saffron are crocin (mono-glycosyl or di-glycosyl polyene esters), crocetin (a natural carotenoid dicarboxylic acid precursor of crocin), and picrocrocin (Monoterpene glycoside precursor of saffranal and product of zeaxanthin degradation), and saffranal (Fig. 1), all contributing not only to the sensory profile of saffron (color, color, taste, and aroma, respectively), but also to the health-promoting properties [20].

Both lipophilic carotenoids and hydrophilic carotenoids have been identified in saffron [1]. The lipophilic carotenoids, lycopene, α-, and β- carotene, and zeaxanthin have been reported in trace amounts [21]. Of the carotenoids, the hydrophilic crocin constitutes approximately 6 to16% of saffron's total dry matter depending upon the variety, growing conditions, and processing methods [8]. Crocin, typically deep red in color, quickly dissolves in water to form an orange colored solution thereby making crocin widely used as a natural food colorant. In addition to being an excellent colorant, crocin also acts as an antioxidant by quenching free radicals, protecting cells and tissues against oxidation [3, 18, 23].

The actual taste of saffron is derived primarily from picrocrocin which is the second most abundant component (by weight), accounting for approximately 1% to 13% of saffron's dry matter [1]. Natural de-glycosylation of picrocrocin will yield another important chemical component, saffranal, which is mainly responsible for the aroma of saffron.

Dehydration is not only important to the preservation of saffron but is actually critical in the release of saffranal from picrocrocin via enzymatic activity, the reaction yielding D-glucose and saffranal, the latter being the volatile oil in saffron. The six major volatile compounds in saffron are saffranal, isophorone, 2,2,6-trimethyl-1,4- cyclohexanedione, 4-ketoisophorone, 2-hydroxy-4,4,6-trimethyl-2,5- cyclohexadien-1-one as well as 2,6,6-trimethyl-1,4-cyclohexadiene- 1-carboxaldehyde [16] but more than 160 additional volatile components have been identified [5]. Of these, saffranal represents approximately 30 to 70% of essential oil and 0.001 to 0.006% of dry matter [5, 16]. Besides its typical spicy aromatic note, saffranal has also been shown to have high antioxidant potential [3, 15] as well as cytotoxicity towards certain cancer cells in vitro [7].

The use of saffron for food coloring and flavoring by the general public is widely accepted throughout the world and by many cultural groups, which may be attributed to the large number of phytochemicals found in saffron. Among these phytochemicals, crocin, picrocrocin, and saffranal are considered the most abundant fractions. Studies clearly indicate that consumption of saffron positively correlates with the amounts of these constituents.

The processing and storage conditions are of great importance because they determine the quality and economic value of the final product [24]. The lack of saffron information has resulted in research focusing precisely on this important issue. The objectives of this paper are to find the relationship between storage times to the various phytochemicals commonly present in saffron and assess the effect of storage conditions on the major characteristics found in this golden spice.

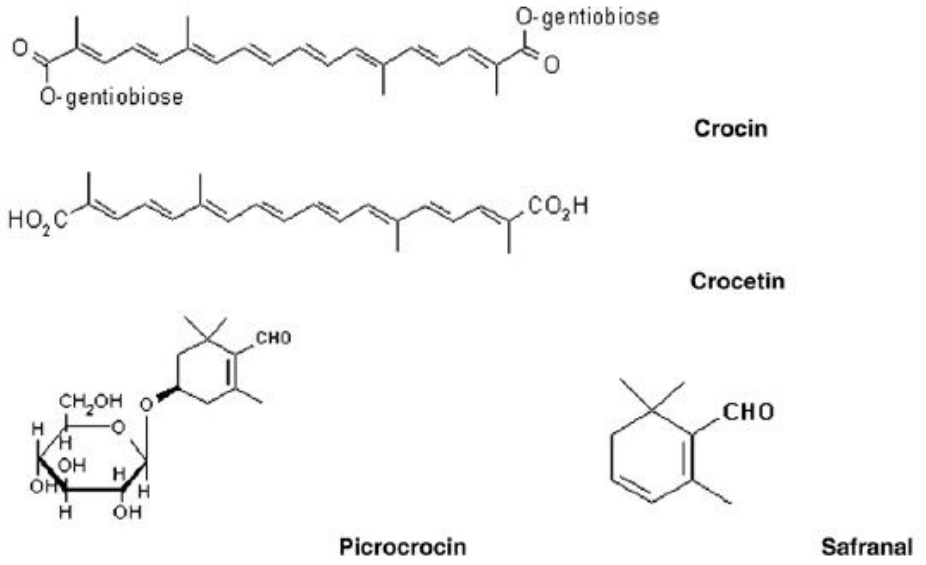

Fig.1. Chemical structures of crocin, crocetin, picrocrocin, and saffranal.

2. MATERIALS AND METHODS

2.1. Materials

A farm near Torbat-Heidarieh (Southern Khorasan province in Iran) was selected for saffron harvesting, the stigmas were separated, dried using Spanish method (55 ^{0}C for 45 minutes until equilibrium moisture content) and packed in polyethylene films and stored at 25 ^{0}C for one year. Experiments carried out every one month intervals.

2.2. Physiochemical analysis

2.2.1. Moisture content

The experiment carried out by using an electrical oven at 100-105 ^{0}C for 16 hours according to Iran national standard NO.259/2 [12].

2.2.2. Aqueous extract

The experiment carried out using Iran national standard NO.1619 [13].

2.2.3. Crocin, picrocrocin and saffranal content

The chemical properties including the coloring strength, aroma and bitterness, were measured using the ISO-3632-2 [14]. In this standard method, the absorbance of the saffron solution was measured at the wavelengths of 440, 330 and 257nm by a UV Visible spectrophotometer after preparation and dilution process.

According to the ISO 3632, the determination was performed using a saffron aqueous solution (50 mg/L), which was filtered through PTFE filter (Millipore, Bedford, MA, USA) with a 0.45 µm pore diameter and their absorbance measured in a Perkin Elmer Lambda 25 UV-Vis spectrophotometer using a quartz cell of 1 cm path length. Then, the coloring strength (*E1% 440nm*), aroma (*E1% 330nm*) and bitterness (*E1% 257nm*) were calculated using the following formula:

$$E^{1\%}_{1cm}\,\lambda_{max} = A*V/m*(100-H)$$

Where λ is wavelength to which is measured the maximum of absorbance, V is the volume of the solvent (mL), m is the saffron mass in gram and H is the sample moisture and volatile matter content. The determination for each sample was carried out by triplicate.

2.3. Microbial analysis

Microbial properties analysed include Total count, Coliform content and Yeast and Mold content all carried out using standard methods [11, 12].

2.4. Statistical analysis

The data were analyzed using SPSS statistical software during the 12-months storage and the mean values were compared with Duncan test. The results were analyzed within the level of 5%. All experiments were done in triplicate.

3. RESULTS AND DISCUSSION

3.1. Physiochemical properties

As can be seen in Fig.2-6 moisture content, water extract, crocin and picrocrocin show a decreasing trend during storage. As moisture content decreased from 7.7 % to 4.8%, water extract from 65% to 58%, maximum crocin absorbance from 250.26 to 208.5 and picrocrocin from 112.9 to 75.5 while saffranal showed an increasing trend and its maximum absorbance increased from 27.24 to 44.62.

These results are in agreement with the results reported by Morimoto (1994), Shoyama (2002), Raina et al. (1999), Alonso et al. (2006) and Bolandi et al. (2006) [17, 19, 1, 4].

Morimoto (1994) had reported that during storage glycosyl esters of saffron degraded under different agents and their contents would decrease in saffron depend on the storage conditions [17]. Shoyama (2002) investigated the crocin decrease during time [22].

Raina et al. (1999) showed that long storing of saffron affects the concentration of colouring and flavor components [19]. Bolandi et al. (2006) studied the effect of temperature (25^0C) and relative humidity (20-30%) during storage on quality properties of saffron and concluded that the colouring strength of saffron decreased but aroma increased during storage and the bitterness did not follow an established pattern [4].

Decrease in crocin content is due to its hydrolysis and converting to free crocetin. Saffranal is from trepens and is in the form of non volatile picrocrocin in fresh saffron but can be degraded during time and free volatile saffranal can be released. Thus there is an inverse relationship between saffranal content and picrocrocin.

By surveying the figures it is observed that the curve slopes are relatively high until July, while after this month a significant variations did not observed in slopes.

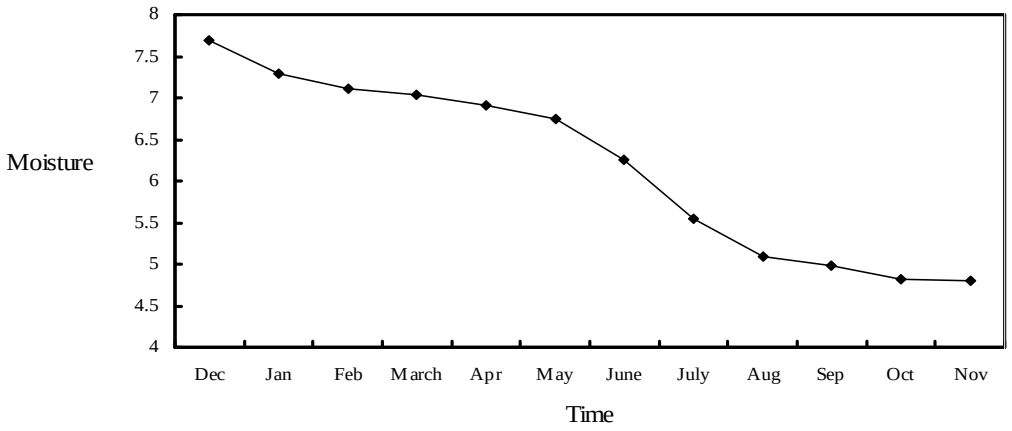

Fig.2. Moisture content variations during one year storage.

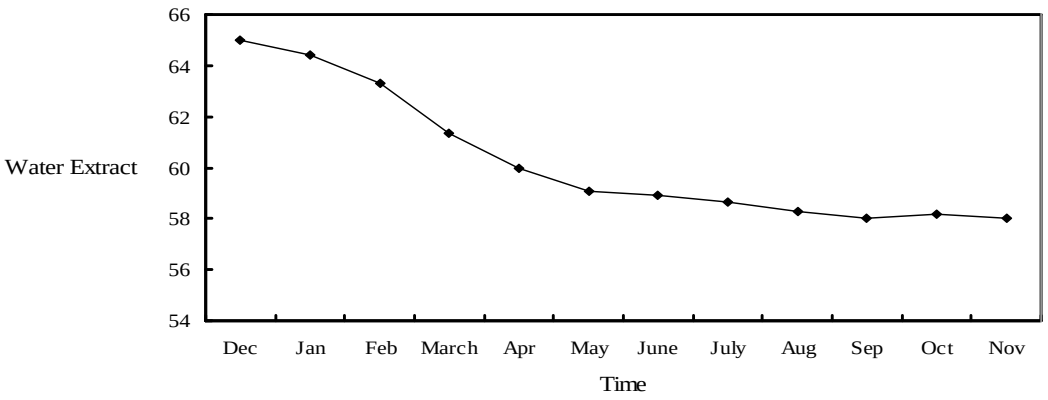

Fig.3. Water extracts content variations during one year storage.

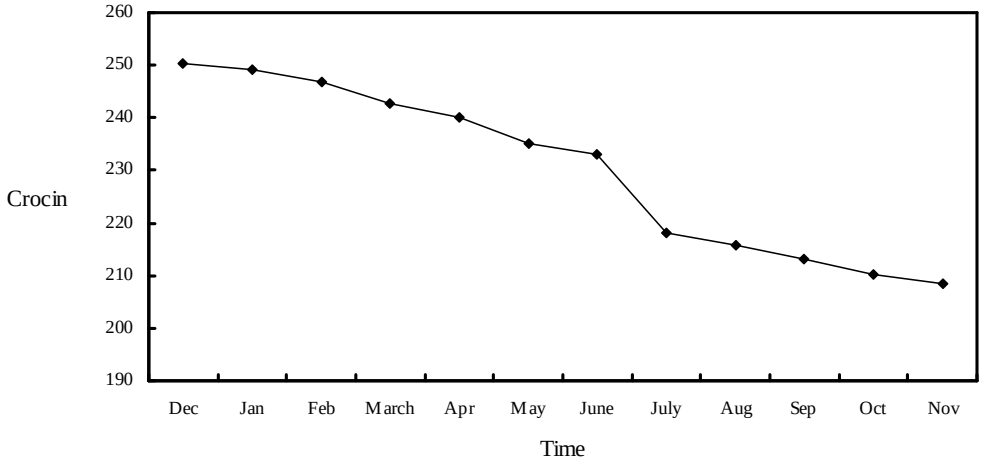

Fig.4. Crocin variations during one year storage.

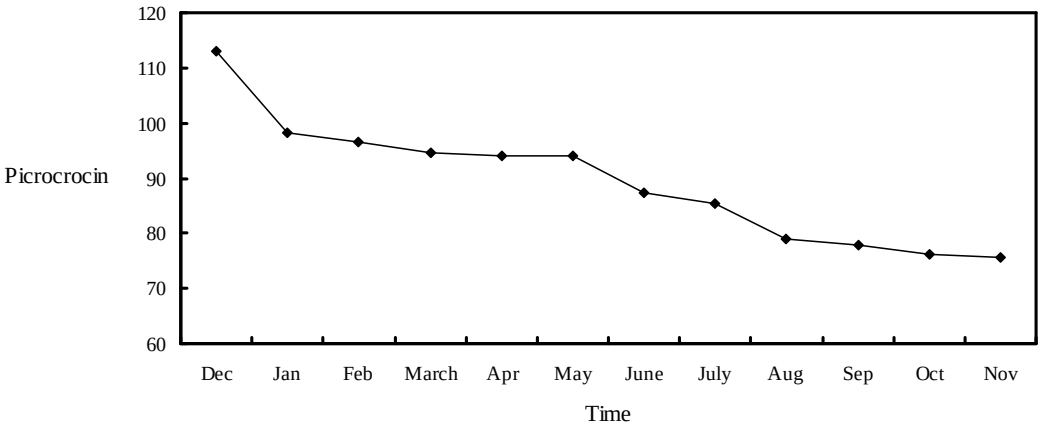

Fig .5. Picrocrocin variations during one year storage.

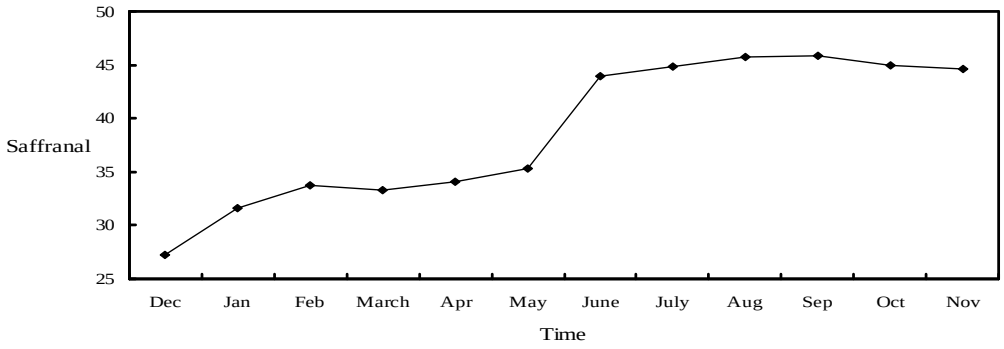

Fig. 6. Saffranal variations during one year storage.

3.2. Microbial properties

As shown in Fig.7-9 all microbial properties including mold content Coliform and Total count decreased during storage time. In these cases Total count decreased from 500 cfu/g to 100 cfu/g, Coliform from 650 cfu/g to 10 cfu/g and mold and yeast content from 100 cfu/g to 10 cfu/g.

Saffranal has an inhibiting effect on the microorganisms growth, thus there is an inverse relationship between saffranal content and saffron total count.

By surveying the variations in microbial properties it is observed that the curve slopes are relatively high until July, while after this month a significant variations did not appear in curve slopes.

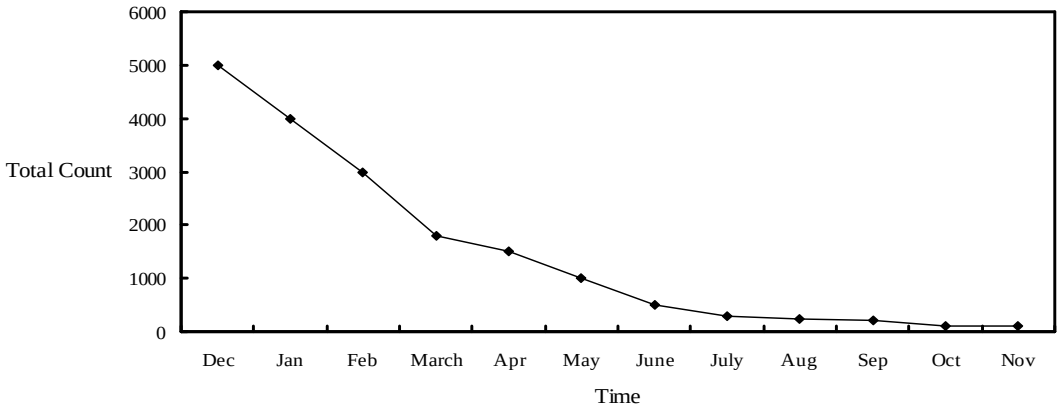

Fig. 7. Total count variations of microorganisms during one year storage.

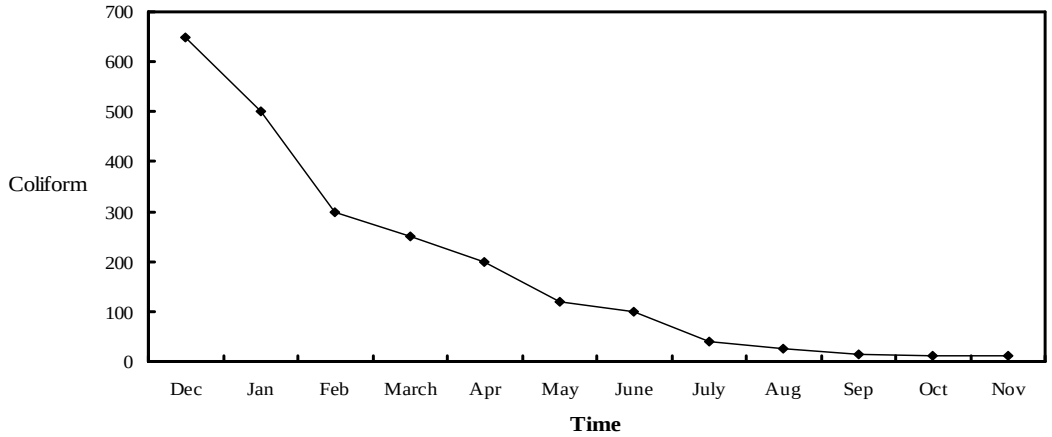

Fig. 8. Coliform content variations during one year storage.

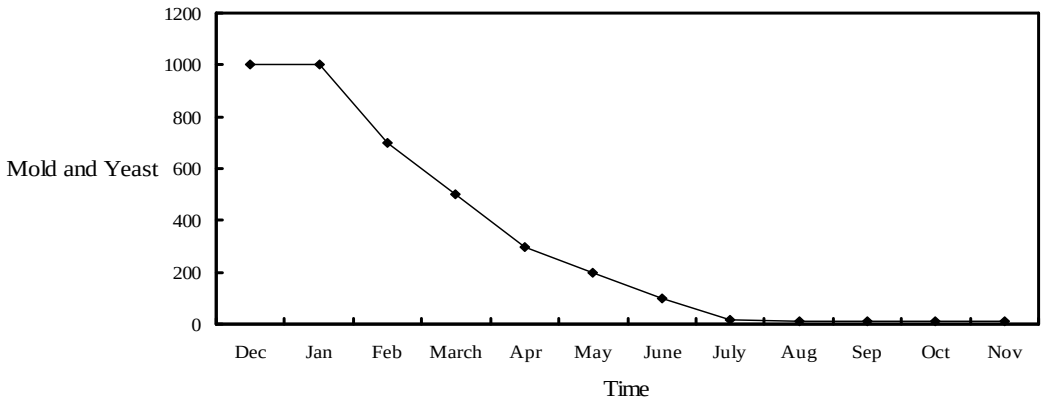

Fig. 9. Mold and yeast content variations during one year storage

4. Conclusions

Moisture content, water extract, crocin, picrocrocin, Total count, Coliform and mold and yeast content decreased during one year storage. However saffranal showed an increasing trend. Analysis of data showed that the largest variation in characteristics studied is until July (from the harvest time, December) and after that a significant variations (at 5 % level) was not observed. Actually, the amount of variation in each of these parameters has been occurred at a higher speed at first eight months and after that it remained almost constant.

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