DRIED FRUITS, ESPECIALLY DATES, INCREASE THE LIFESPAN OF *CAENORHABDITIS ELEGANS*

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Abstract

The effects of five commercially available dried fruits on the longevity of nematodes were investigated. All five dried fruits significantly increased the lifespans relative to the control. In particular, dates extended the lifespan significantly more than the control and the other four dried fruits. The mean lifespan and maximum lifespan of the nematodes that consumed dates were 40.9 and 21% longer than those of the control group, respectively. The total concentration of polyphenol compounds was measured to investigate whether the lifespan-extending effect of dates was due to antioxidants. The results showed that the polyphenol contents of dates and prunes were higher than those of the other dried fruits. However, there was no significant difference in the total polyphenol concentrations between dates and prunes. Therefore, dates may contain substances other than polyphenol-related compounds that are responsible for their lifespan-extending effects, or which may promote the activities of polyphenols.

Keywords: *dried fruits, life extension, antioxidants, prune, date, Caenorhabditis elegans*

1. INTRODUCTION

Fruits are eaten as a side dish or delicacy because of their bright colors and distinctive flavors. In terms of composition, they contain moderate amounts of sugars and acids and are a significant energy source. They are also sources of minerals, vitamin C, and dietary fiber. Fruits are often eaten raw, but their high water content, respiration, and transpiration cause them to lose their freshness quickly. In Japan, the Ministry of Health, Labor and Welfare and the Ministry of Agriculture, Forestry, and Fisheries have developed a Japanese Food Guide Spinning Top that recommends consuming at least 200 g of fruit per day (i). However, actual fruit intake usually falls short of this recommendation. The 2019 National Health and Nutrition Survey revealed an average daily intake of only 98.9 g of fruit per person (ii).

To enhance their preservation and accessibility, many fruits are processed into juice and jam products. Drying is one of the processing methods, which reduces the weight and volume of fruit significantly, making it convenient for transportation and storage. Dried fruits have become more of a specialty item due to the change in texture and the improvement in sweetness.

Furthermore, dried fruits have the advantages of being inexpensive, readily available, easily preserved, and easily distributed, and can easily be consumed as a substitute for raw fruits. Various fruits are processed into dried fruits, including raisins, prunes, figs, and apricots, which are widely distributed. Moreover, dried fruits are concentrated with nutritional and functional ingredients such as vitamins, minerals, dietary fiber, and polyphenols (also known as antioxidants). Dried fruits contain various kinds of polyphenols. For example, raisins are rich in flavonoids, such as quercetin and kaempferol, and hydroxycinnamic acids, such as caffeic acid and *p*-coumaric acid (Chiou, A *et al.*, 2007). Chlorogenic acids, which are derivatives of caffeic acids, account for about 94% of the polyphenols in prunes

(Nakatani, N *et al.*, 2000). Prunes and apricots show high values in the evaluation of antioxidant properties of plant foods due to these polyphenols (iii).

Although dried fruits contain high concentrations of bioavailable sugars, they cause a slow rise in blood glucose and insulin levels. Thus, they are often referred to as low-GI (glycemic index) foods with GI values between 30 and 60 (Kim, Y *et al.*, 2008, Miller, CJ *et al.*, 2002, Viguiliouk, E *et al.*, 2018). This effect is believed to be due to the abundant dietary fiber and polyphenols contained in dried fruits (Björck, I & Elmståhl, HL, 2003, Johnston, KL *et al.*, 2003, Widanagamage, RD *et al.*, 2009, Zunino, SJ, 2009). Thus, dried fruits are reported to have many functional properties.

Recently, foods with anti-aging and biological defense-activating effects have attracted substantial interest. One method of evaluating foods is to use *Caenorhabditis elegans,* which has been analyzed at the genetic level and used as a model animal for longevity research. There are numerous reports demonstrating that antioxidants are involved in the life extension of *C. elegans* (Arif, MU *et al.*, 2022, Liu, L *et al.*, 2021, Martel, J *et al.*, 2020, Mudd, N & Liceaga, AM, 2022, Okoro, NO *et al.*, 2021). Therefore, we evaluated the effects of commercially available dried fruits on the longevity of *C. elegans*.

2. MATERIALS AND METHODS

2.1. Food samples

All five dried fruits used in this study were purchased from local markets in Hiroshima, Japan. When purchasing dried fruits, we chose products with no added sugars, preservatives, or other additives. Among the dried fruits, prunes and dates contained seeds. Therefore, the seeds were removed prior to testing. The dried fruits were then chopped into 1 cm cubes. The chopped dried fruits were frozen in a freezer at -25°C for 24 hours. The frozen dried fruits were finely ground using a crush milling machine (Iwatani Corporation IFM-720G, Japan) and added to the nematode growth medium (NGM) plate (3 g/L NaCl, 3 g/L peptone, 20 g/L agar, 5 µg/mL cholesterol, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 25 mM potassium phosphate buffer (pH 6.0)) at a rate of 0.4 g/100 mL.

2.2. Caenorhabditis elegans cultivation

Caenorhabditis elegans strain N2 (wild type) was used as the experimental animal. Each *C. elegans* was kept at 20°C on the NGM plate supplemented with *Escherichia coli* OP50, which was used as a food source (Brenner, S, 1974). After 6 hours, the nematodes were removed, leaving only eggs. This point was considered to be the 0th day of measurement (Ogawa, T *et al.*, 2022).

2.3. Lifespan assay

The day after the synchronous incubation was designated as day 1, and nematodes were kept at 20 °C. On day 4, 200 µg/mL of 5-fluoro-2′-deoxyuridine (FUdR)(FUJIFILM Wako Pure Chemical Corporation, Japan) was added to the NGM plates and incubated for another day. In addition, NGM plates containing 200 µg/mL FUdR and spread with *E. coli* OP50 were stored in a refrigerator at 20 °C beforehand. On day 5 of incubation, 30 nematodes were transferred to a new NGM plate. For the plates used to investigate the effects of dried fruits, 0.4 g (dry matter weight) of each dried fruit was added per 100 mL of NGM medium when the NGM plates were prepared. After that, every 2 days, the nematodes were judged alive or dead based on the presence or absence of nematode responses to physical stimulation (Hosono, R, 1978, Mitchell, DH *et al.*, 1979).

2.4. Measurement of total phenolic compounds

Dried fruit powder (0.2 g) was extracted with 10 mL of 80% methanol (Ishiwata, K *et al.*, 2004). The total concentration of phenolic compounds in the methanolic extract of dried fruits was measured according to the Folin–Ciocalteu method (Bonoli, M *et al.*, 2004, Chun, OK *et al.*, 2005, Singleton, VL, 1965). The standard solution was 10–50 µg/mL gallic acid. One milliliter of sample solution (gallic acid standard solution or dried fruit extract extracted with 70% methanol) was added to 5 mL of 10 % Folin–Ciocalteu reagent and reacted for 3 min. The reaction was stopped by adding 4 mL of 7.5 % sodium carbonate, and the absorbance was measured at 756 nm. The total polyphenol content was determined as gallic acid equivalent using a standard curve of gallic acid and expressed as mg of gallic acid equivalent per 100 g of dried fruit powder.

2.5. Statistical analysis

All experiments were performed in triplicate unless otherwise specified. For *C. elegans* survival curves, Kaplan–Meier survival curves were generated, and the data were analyzed by the log-rank test using EZR on R commander (programmed by Y. Kanda) Ver.1.55 (Kanda, Y, 2013).

The mean lifespan (MLS) of *C. elegans* was estimated using the following formula (Wu, D *et al.*, 2006).

$$MLS = \frac{1}{n}\sum_j \frac{x_j+x_{j+1}}{2} d_j \quad \cdots\cdots\cdots\cdots\cdots\text{Eq. 1}$$

Where j is the age category (day), d_j is the number of nematodes that died in the age interval (x_j, x_{j+1}), and n is the total number of nematodes. The standard error (SE) of the MLS estimate was calculated using the following equation.

$$SE = \sqrt{\frac{1}{n(n-1)}\sum_j (\frac{x_j+x_{j+1}}{2} - MLS)^2 d_j} \cdots\cdots\cdots\cdots\cdots\text{Eq. 2}$$

The maximum lifespan was calculated as the MLS of the top 15 % longest-living nematodes from each group (Kashima, N *et al.*, 2012, Komura, T *et al.*, 2013).

3. RESULTS AND DISCUSSION

The numbers of surviving *C. elegans* cultured in NGM medium containing various ground dried fruits were counted. To statistically evaluate the effect of dates on nematode survival, the survival curves of the nematodes were calculated using the Kaplan–Meier method, and the differences in survival rates among the groups were compared using the log-rank test. Figure 1 shows the survival curves calculated using the Kaplan–Meier method.

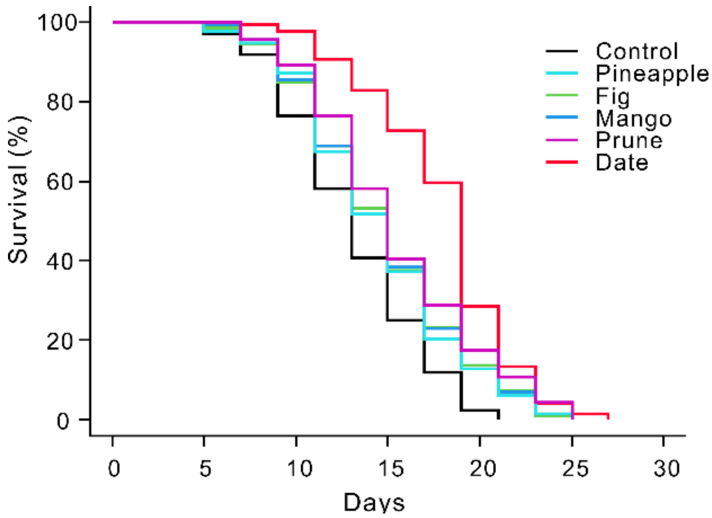

Fig. 1. Effect of dry fruits on the life span of *C. elegans*.

Representative Kaplan-Meier survival curves of *C. elegans* with or without dry fruits.

As shown in Fig. 1, all the nematodes cultured in a medium containing dried fruit had an extended lifespan. In particular, when dates were added, there was a significant increase in the number of surviving nematodes in the 50% survival region.

The average and maximum lifespans were calculated for the results in Fig. 1, and are shown in Table 1.

Table 1. Lifespan analysis data

Sample	Number of nematodes	Mean life span (days)	Maximum Survival time (days)
Control	212	12.07±0.3382	18.88
Pineapple	212	13.53±0.3014	23.06
Fig	218	13.66±0.3037	21.69
Mango	213	13.68±0.3000	21.81
Prune	222	14.42±0.3087	21.91
Date	217	17.00±0.2648	23.48

As shown in Table 1, the MLS was increased by 12.2, 13.2, 13.4, 19.5, and 40.9 % in the pineapple-, fig-, mango-, prune-, and date-added groups, respectively, compared with the control group. Notably, the lifespan of the date-added group was significantly longer than that of the control group and the other dried fruit-added groups. However, the maximum lifespan of the date-added group was only 21% longer than that of the control, which was less dramatic than the difference between the MLSs. Table 2 shows the results of the significant difference tests among the groups.

Table 2. Pairwise comparisons using logrank test of dry fruits

	Control	Pineapple	Fig	Mango	Prune
Pineapple	<0.001***	—	—	—	—
Fig	<0.001***	0.748	—	—	—
Mango	<0.001***	0.764	0.983	—	—
Prune	<0.001***	0.031**	0.061	0.060	—
Date	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

[a]*, ** and *** indicate a statistically significant difference at P values of <0.05, <0.01, and < 0.001, respectively.

The results in Table 2 reveal that all dried fruit groups significantly increased the life expectancy compared with the control group. In addition, the date-added group exhibited a significantly longer lifespan than the control group and the other dried fruit groups.

Raw fruits are known to contain various antioxidants in relatively high concentrations (Shibamura, A *et al.*, 2009, Sun, J *et al.*, 2002, Vinita *et al.*, 2022), which are apparently maintained after drying (Chang, SK *et al.*, 2016, Ishiwata, K *et al.*, 2004, Lutz, M *et al.*, 2015, Mishra, N *et al.*, 2010, Ouchemoukh, S *et al.*, 2012, Reddy, CVK *et al.*, 2010). Furthermore, antioxidants are known to extend the lifespans of *C. elegans*. Several reports have demonstrated the lifespan extension of *C. elegans* using fresh fruits (Ajagun-Ogunleye, OM *et al.*, 2021, Arif, MU *et al.*, 2022, Coppari, S *et al.*, 2021, Jabeen, A *et al.*, 2020, Pallauf, K *et al.*, 2017, Wang, J *et al.*, 2020, Wilson, MA *et al.*, 2006), and those studies reported that antioxidants play a primary role in extending the lifespan. Therefore, we also measured the content of polyphenolic compounds, which are indicators of the antioxidant capacity of the commercially available dried fruits that were used in this study. The results are shown in Fig. 2.

As shown in Fig. 2, the dried fruits contained large quantities of phenolic compounds. In particular, dates and prunes contained significantly more than the other dried fruits. However, there was no significant difference in the content of phenolic compounds between dates and prunes.

The date palm (*Phoenix dactylifera* L.) is the most popular fruit in the Middle East and North Africa. It is consumed widely and has been used in traditional medicine. The date is said to be a God-given plant in the Arab world, and some authorities believe that the tree of life in the story of the Garden of Eden was a date palm (Nixon, RW, 1951, Qureshi, R *et al.*, 2006).

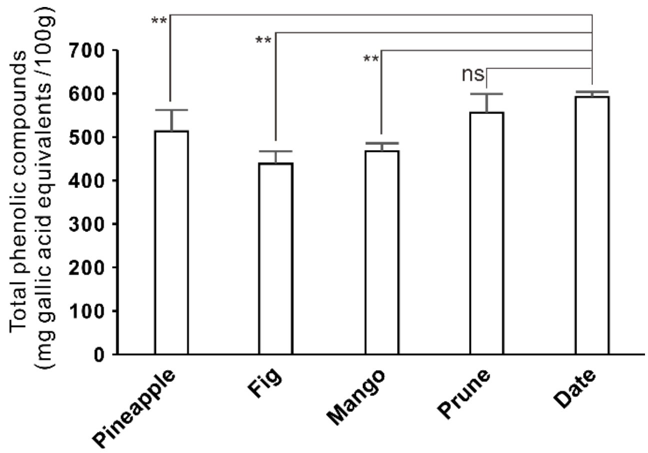

Fig. 3. Concentration of total phenolic compounds

** indicates a statistically significant difference at *P* values of <0.01. ns means not significant (*P*>0.05).

For these reasons, many studies have been conducted to determine the functional ingredients in dates. Al-Shwyeh reported that dates contain various antioxidative ingredients such as carotenoids, flavonoids, tocopherols, and resveratrol (Al-Shwyeh, H, 2019). Jabeen and coworkers revealed that date seeds promote lifespan-extending effects in *C. elegans* (Jabeen, A *et al.*, 2020). Unlike our study, they used date seeds and focused on their antioxidant properties. They reported that date seed extract extended the mean and maximum lifespans of *C. elegans*. Studies with date seeds also suggested that syringic acid, an antioxidant found in high amounts in date seed extracts, is related to prolonging nematode lifespans (Jabeen, A *et al.*, 2020). In our study, dates were shown to have a similar amount of phenolic compounds, an indication of antioxidant content, compared to prunes. Previous studies have also reported that the lifespan-extending effects of prunes are related to their antioxidant capacity (Coppari, S *et al.*, 2021). However, we observed that dates had the same amount of phenolic compounds as prunes and a much better lifespan extending effect. This result suggests that antioxidants are not the only lifespan-extending component of dates or that dates support the bioavailability of antioxidants. Another report implicated phenolic compounds in the anti-aging effects of fruits and vegetables (Liu, L *et al.*, 2021).

Moreover, reports have shown that glycoproteins and polysaccharides, which are not phenolic compounds, are related to lifespan extension (Mudd, N & Liceaga, AM, 2022, Okoro, NO *et al.*, 2021). Additionally, multiple substances in blueberries and oranges may be involved in lifespan extension. Therefore, substances other than phenolic compounds may contribute significantly to the lifespan extension provided by dates. In future studies, we intend to identify the critical components responsible for the lifespan-extending effects.

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