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RESEARCH ARTICLE

Development and characterisation of yoghurt incorporated with Dates (*Phoenix Dactylifera Linn*) extract

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Abstract: Nowadays, functional dairy products gain more acceptance in the market because of the additional health and nutritional benefits they provide to the consumer. Value addition of dairy products like yoghurt often augments the market value as well as functionality of the product. Dates (*Phoenix dactylifera Linn*) are natural source of phytochemicals like phenolic acids and flavonoids which are proved to have strong anti-oxidant activity. In this study, the effect of addition of dates extract into yoghurt milk was studied. Hot water extract of dates fruit was added to the yoghurt at different levels (30%, 32%, 34%, and 36%) and the optimum level of addition was selected on the basis of sensory scores obtained from 5 semi trained judges. Significant differences ($p<0.01$) were found in the sensory scores of different treatments and the overall highest score was obtained for yoghurt with 34% dates extract. The product selected was subjected for physico chemical analysis and the findings were: fat 2.85%, protein 3.8, total solids 15.88 and acidity 0.79% Lactic acid (LA). The total phenolic content of the product was measured in terms of milligrams of Gallic acid equivalents (GAEs) per 100 grams of sample (mg GAE/g of sample) and was found to be 59 mg GAE/100g. The phenolic contents in the product was proved to impart antioxidant activity in the yoghurt as the IC 50 value; the half maximal inhibitory value, was found to be 19.99mg/mL of the sample.

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Keywords: Anti-oxidant activity, Dates extract, Yoghurt, Total phenolic content

Introduction

Rising interest in healthy eating habits has led to a new array of food products on the market which, despite of providing nutritional benefits, adds to improvement in health and reducing the risk of certain diseases. Internationally accepted dairy products like yoghurt can act as a medium for the incorporation of functional components like poly-phenolic compounds (Chouchouli et al. 2013). Yogurt is obtained by the fermentation of milk by *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus*. It is a good source of essential micro and macro nutrients including bioactive peptides, with good anti-oxidant properties produced during fermentation (Granato et al. 2010)

Yoghurt is considered to be a product with high functionality owing to the presence of living microorganisms such as bifidobacteria, lactic acid bacteria (LAB), streptococci or their combinations. Fermented milk products comprising yoghurt are proven to be good sources of minerals like calcium and potassium as well as proteins (Dimidi et al. 2019). Moreover, yoghurt also plays a potential role in decreasing intestinal disorders and chronic diseases (Balakrishnan et al. 2016).

However, plain yogurt contains very less phenolic and anti-oxidant compounds and their impact on human health is of little significance. In order to improve the nutritive value of yoghurt, it is often added with antioxidants from natural sources which provides additional health benefits to it. Hence, a number of attempts to prepare yoghurts fortified with natural antioxidant-rich extracts have been done by the functional food industry (Chouchouli et al. 2013). The potential useful components of functional ingredients for this fortification are carotenoids, Vitamin C, flavonoids, Vitamin E and other polyphenols (John and Singla 2021). Plants synthesise natural functional compounds including polyphenols as secondary metabolites as a response to environmental stress. The phenolic components in foods offer many possible beneficial effects on human health and therefore are the subject of increasing scientific interest. Various studies

have shown that diets rich in plant polyphenols, when consumed for a long term contributed to the prevention against the development of cancers, osteoporosis, neurodegenerative diseases, diabetes, and cardiovascular diseases (Pandey and Rizvi 2009). Yoghurt added with natural extracts or concentrates of fruits like dates, grapes, passion fruit etc, are known to have good anti-oxidant activity and poly phenol contents (Amerinasab et al. 2015). Dates fruit (*Phoenix dactylifera L.*) is usually available in market as ready-to-use, semi-finished products or products like date juice, date syrup, date spread and date liquid sugar (DLS). The dried dates fruit contain a high percentage of carbohydrates (total sugars, 66.1 to 88.6g/100g), fat (0.1 - 1.4g/100g), protein (1.6–3g/100g), energy (258 –344kcal/100g), ash (1.3-1.9g/100g). Dates also contain good number of antioxidants, primarily carotenoids and phenolics. It also contains a considerable amount of dietary fiber (8 g/100 g), antioxidants (80400 µmol/100 g) and phenolics (3942 mg/100 g) (Al-Farsi and Lee 2008). The unsaturated fatty acids existent in dates are linoleic, oleic and linolenic acids. Dates also contain minerals like magnesium, copper, selenium and potassium and vitamins like Vitamins C and B-complex.

The objective of this study was to fortify a yogurt with natural extract of dates fruit in order to improve the functionality of the product as well as to improve the sensory attributes of the yoghurt. The product was prepared by adding the hot water extract of dates fruit before inoculation which was followed by incubation after which the sensorial and quality attributes of the formulated product was compared with plain yogurt.

Materials and Methods

Preparation of Dates extract

Good quality dates were procured from the local market and was cleaned properly. Dates, after removing the seeds, were crushed using a mortar and pestle. The crushed dates was infused with 2 times of hot water and then stirred for 2 hours. The mix was allowed to stand for overnight in cold place to obtain the complete extract. The dates-water mix was then boiled until the tissue softened and the extract was obtained by squeezing the mix using sterilised muslin cloth. The hot water extract of dates was bottled and used.

Preparation of Dates incorporated yoghurt

Cow milk was procured from the Kerala Veterinary and Animal Sciences University Dairy Plant, Mannuthy, Thrissur. Yoghurt culture was procured from Dairy Microbiology Laboratory, Kerala Veterinary and Animal Sciences University. The total solids content of the milk was standardised to about 13% by the addition of skim milk (3% fat and 10% SNF). Preliminary trials were conducted to select the maximum and minimum levels of incorporation of the dates extract into the milk. Based on these preliminary trials, four levels (30%, 32%,34% and 36% of the milk) were considered for further sensory analysis in 4 replications. The milk used to make yoghurt was heated to 90p C for 15 minutes followed by cooling to 42°C. Dates extract was added to milk at different levels followed by inoculation with yoghurt culture, 1:1 mixture of *S. Thermophiles and L. bulgaricus* and then the cultured mix was incubated at 42°C for 4 hours.

Sensory evaluation

Sensory evaluation of the samples was carried out by a panel of 5 semi trained judges. The parameters of study were the color and appearance, body and texture, flavour, acidity and overall acceptability. The sensory evaluation was carried out based on 9-point Hedonic scale in which a score of 1 represented 'dislike extremely' and score of 9 represented 'like extremely'. The samples for analysis were presented before the judges after suitable marking. The judges were provided with a room with good lighting and appropriate facilities. The selection of optimum product from all the treatments was done based on the sensory scores obtained.

Texture Profile analysis

Texture analyser (Shimadsu, Model EZ-X series, Japan) fitted with a load cell of 200N and a cylindrical probe (25.4 mm in diameter) connected to the software TRAPEZIUM X was used to determine the texture profile of yoghurt. Samples were kept at 25 °C prior to TPA analysis. TPA was performed by using a probe to compress twice and achieve a penetration of 10 mm at a velocity of 1 mm/s. Hardness, adhesiveness, cohesiveness and gumminess were determined from TPA by using software. All measurements were carried out in triplicate for each sample.

Proximate composition analysis

The milk used for yoghurt preparation as well as the dates incorporated yoghurt were subjected for proximate analysis. The total solids and protein content of the samples were determined as per IS: 1479 (part-II), 1961.The fat content was determined by using standard Gerber method as per IS: 1224 (part-I), 1977 and the acidity was estimated according to IS: 1479, (part–I), 1960.

Total phenol content and anti-oxidant activity

Total polyphenols in the dates extract and the dates yoghurt were determined by employing the Foline Ciocalteu assay, as described by Arnous et al. (2002). Gallic acid was taken as the reference standard, and results were expressed as mg gallic acid equivalents (GAE) per g fresh weight (mg GAE/g of sample).

The DPPH assay was conducted for the determination of anti-oxidant activity in the developed product. The assay measures the reducing ability of the anti-oxidants present in the product towards the DPPH radical. A decrease in DPPH radical absorption

is indicated by a change in hue from purple to yellow. This shows that antioxidants in a mixed solution interact with free radicals. The anti-oxidant activity of dates yoghurt was determined using the procedure given by Brand et al. (1995). A 100ul aliquot of the sample was vortexed with 2.9ml of 60Um DPPH (2, 2- diphenyl-1-picrylhydrazyl) solution in methanol. After 30 minutes in the dark, the absorbance of the mixture was measured at 517 nm. As a control, methanol was employed. The IC (Inhibition Concentration) 50 values for antioxidant activity were given in milligrammes per litre. The concentration of anti-oxidant in the test solution was related to the decrease in DPPH absorbance compared to blank determined spectrophotometrically at 516nm. The percentage of inhibition of DPPH oxidation was calculated according to the formula:

$$\text{DPPH Scavenging effect (\%)} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Color characteristics

MiniScan EZ spectrophotometer was used for the measure of color parameters of the yoghurt samples. Both the control and optimized samples of yoghurt were tempered to room temperature. The samples were filled in petriplates for taking measurement. The color characteristic parameters such as 'L*', 'a*' and 'b*' values were noted; The colour parameter 'L* ' is a measure of lightness or luminance, which ranges from 0 to 100 [L* = 0 (black) and L* = 100 (white)] and 'a* ' and 'b* ' are the two chromatic components, which range from -120 to -120 [a* = -120 (green) and a * = 120 (red)] and [b* = -120 (blue) and b* = 120 (yellow)] respectively.

Statistical analysis

The results were analysed using SPSS v.16.0 for windows software. The sensory scores obtained for different treatments while optimising the product, were subjected to Kruskal – Wallis test followed by Mann-Whitney u-test. The data obtained during compositional analysis of the optimised dates yoghurt and the control was compared using independent t test.

Results and Discussion

Sensory evaluation

Preliminary trials were conducted by incorporating dates extract at different concentrations in milk intended for the preparation of yoghurt. The minimum and maximum level of incorporation of dates extract in milk was selected as 30% and 36% respectively. The sensory scores obtained for different treatments were statistically analysed and given in Table 1 and the graphical comparison of the sensory scores are given in figure 1. The treatments T1 depicts 30% dates extract, T2 depicts 32% extract, T3 represents 34% extract and T4 expresses 36% extract in milk.

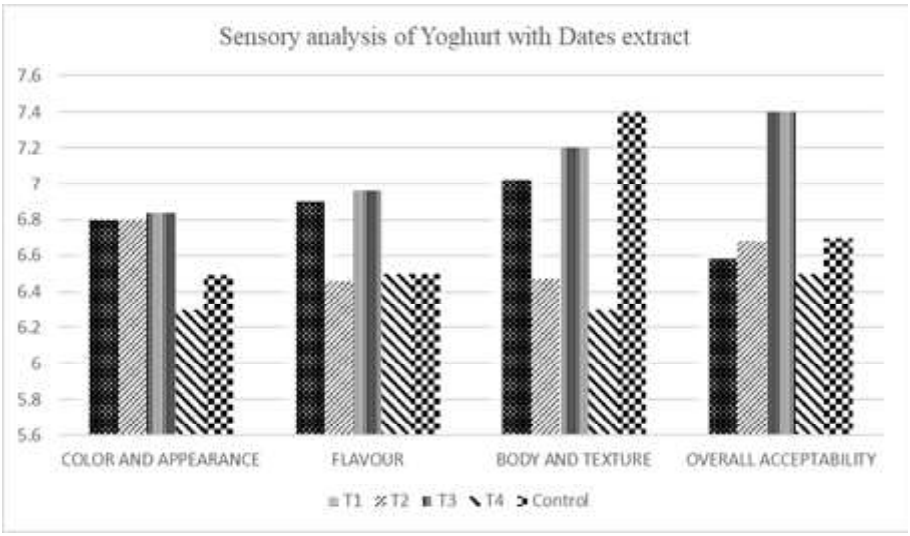

Fig. 1 Sensory analysis of dates incorporated yoghurt and the control sample

T1 - 30% dates extract added yoghurt, T2- 32% extract added yoghurt, T3 -34% extract added yoghurt and T4-36% extract added yoghurt

Table 1 Sensory analysis data of treatment samples and control yoghurt

Parameters	Sensory scores					
	T1	T2	T3	T4	Control	Chi square value
Color and appearance	6.8±0.131^{a}	6.8±0.077^{a}	6.84±0.092^{a}	6.30±0.122^{b}	6.49±0.032^{b}	20**
Flavour	6.9±0.015^{a}	6.46±0.128^{b}	6.96±0.081^{b}	6.5±0.058^{b}	6.50±0.158^{b}	16.9**
Body and Texture	7.02±0.159^{a}	6.47±0.012^{b}	7.02±0.122^{a}	6.3±0.111^{b}	7.40±0.100^{a}	12.2**
Overall Acceptability	6.58±0.073^{a}	6.68±0.096^{a}	7.4±0.100^{b}	6.5±0.158^{a}	6.70±0.122^{a}	22.6**

**- Significant at one per cent level ($p<0.01$), *- Significant at five per cent level ($p<0.05$), a-b Figures in a row bearing different superscript differ significantly, T1 - 30% dates extract added yoghurt, T2- 32% extract added yoghurt, T3 -34% extract added yoghurt and T4-36% extract added yoghurt. Figures are the mean ± standard error of sensory scores by five semi trained judges in four replications

The chi square values obtained in the study were found to be significant ($p<0.01$) indicating significant differences between the samples with respect to all the attributes. Highest score for flavour was secured by the yoghurt with 34% (T3) dates extract as compared with other treatments and control. However, the control sample secured the highest score for body and texture as the treated samples had less firm body compared to it. However, yoghurt with 34% dates extract had comparable scores to that of the control yoghurt. The scores obtained for color and appearance was moderately same for all the treated samples except for yoghurt with 36% dates extract. From the overall sensory scores obtained, yoghurt with 34% dates extract (T3) was selected as the optimum product and was subjected for further physicochemical analysis.

Amerinasab et al. (2015) studied the sensory characteristics of yoghurt added with date liquid sugar (DLS), obtained from refined dates syrup and concluded that increasing concentration of DLS in yoghurt beyond 6 per cent level resulted in decreasing firmness of the product. This decrease in firmness was found to be associated with the higher rate of syneresis and lower viscosity, caused by the addition of higher amounts of DLS.

Texture Profile analysis

It is proved that the structure of food has significant impact on various aspects, including functionality, texture and appearance. The composition and micro structure of the protein network determine the textural and rheological characteristics of fermented dairy products. (Delikanli and Ozcan, 2017, Kose et al. 2018). The data obtained for textural analysis (Table 2) showed that the textural parameters of all the treatments differ significantly with that of the control yoghurt. A notable difference was observed in the hardness of the treatment samples as compared to the control which was found to be decreasing with increase in the level of dates extract. This descending manner of hardness can be associated with the reduction in total soilds. Various studies also reported that, a decrease in hardness may be directly related to the fat content of yoghurt. (Berber et al. 2015, Lucey, 2014). Considering the dilution occurred on addition of dates extract, control yoghurt had higher percentage of fat which may have contributed to the much higher hardness than the treated samples. The hardness of yoghurt was found to fall within the range of 0.22N to 1.54N for traditional yoghurt and 0.94N to 224.62N for industrial yoghurt samples (Kose et al. 2018). Cohesiveness in semi-solid materials refers to the extent to which product sustains a second deformation in compared to how it reacted to the initial deformation (Ashu and Pradyuman, 2013). Cohesiveness of the yogurt showed a significant decrease with increasing levels of dates extract. This decrease may be caused by the dilution occurring due to the incorporation of dates extract. Reduced cohesion may be a result of the system's decreased viscosity (Mudgil et al. 2017).

Adhesiveness is defined as the negative force area for the first bite and depicts the effort required to overcome the attraction forces between the surface of a food and the surface of other materials with which the food comes into contact (Kasapis, 2009). It is the force required to remove the food material that adheres to the mouth while eating (Ganesh, 2006). The control yoghurt and treatment 1 (T1) had minimum adhesiveness as compared to the other treatment samples. The more negative the value of adhesiveness, the more "sticky" is the sample (Armero and Collar, 1997). A significant difference was found in the gumminess values of control yoghurt and the treatment samples. The force required to break down a semisolid food into a state suitable for ingesting is known as gumminess, which is measured by multiplying hardness and cohesiveness. (Yang and Li, 2010, Najgebauer-Lejko et al. 2015). As in the case of hardness, the treatment samples had a very lesser value of gumminess when compared with the control yoghurt. A similar decreasing trend in gumminess value was observed by Mendes et al. (2019) in yoghurt incorporated with Yakon syrup and cashew apple extract. As the level of incorporation increased, the hardness and gumminess value were decreased.

Proximate composition analysis

The cow milk used for the preparation of yoghurt was standardised to 13% total solids. The acidity was found to be 0.14 per cent lactic acid. The chemical quality of the optimised sample with 34% dates extract and control yoghurt is given in Table 2. The total soluble solids (TSS) content of the dates extract was found as 58p Brix using a hand refractometer. The inclusion of this dates extract may be responsible for the significant difference ($p<0.01$) in total solids between the dates yoghurt and the control. A significant difference ($p<0.01$) was observed in the fat content owing to the dilution occurring in the sample with addition of dates extract. Jung et al. (2016) observed similar

Table 2 Textural quality of treated samples of dates incorporated yoghurt

	Control	T1	T2	T3	T4	F value
Hardness (N)	0.98±0.009^a	0.284±0.142^b	0.271±0.126^b	0.260±0.120^c	0.215±0.107^d	2428.6**
Cohesiveness	0.543±0.271^a	0.527 0.263^b	0.526 0.263^b	0.48 0.015^c	0.477±0.238^c	43.8**
Gumminess	0.532±0.002^a	0.150±0.068^b	0.142±0.066^b	0.125±0.016^c	0.103±0.059^c	95.3**
Adhesiveness (J)	-0.0003±0	-0.0003±0	-0.0001±0	-0.0001±0	-0.0001±0	

**- Significant at one per cent level ($p<0.01$), *- Significant at five per cent level ($p<0.05$), a-b Figures in a row bearing different superscript differ significantly, T1 - 30% dates extract added yoghurt, T2- 32% extract added yoghurt, T3 -34% extract added yoghurt and T4-36% extract added yoghurt. Figures are the mean ± standard error of sensory scores by five semi trained judges in four replications

Table 3 Chemical quality of yoghurt incorporated with 34% dates extract and control

	Chemical composition		
	Dates yoghurt	Control	t value
Fat (%)	2.85±0.022	3.08±0.0.083	5.2**
Protein (%)	3.8±0.141	3.91±0.008	1.7^{ns}
Total solids (%)	15.88±0.083	15.48±0.083	7.5**
Acidity (% LA)	0.79±0.005	0.63±0.015	9.95**

Figures are mean ± standard error of three replications, *-Significant at one per cent level ($p<0.01$), ns – non significant

Table 4: Color characteristics of dates incorporated yoghurt and the control sample

Parameter	Dates Yoghurt (T3)	Control sample	t value
L*	82.04±0.160	89.02±0.045	-41.9**
a*	0.62±0.003	2.41±0.003	-166.7**
b*	17.8±0.063	14.66±0.031	44.4**

Figures are mean ± standard error of three replications, *-Significant at one per cent level ($p<0.01$), ns – non significant

decrease in the fat content and increase in total solids content in a study conducted on yoghurt added with red ginseng extract. Whereas the protein content in both the control and dates yoghurt tend to remain same with no significant difference ($p<0.01$). Similar insignificant decrease in protein content was observed by Gad et al. (2010) in a study conducted in yoghurt added with date palm syrup and skim milk.

Similarly, acidity of the control yoghurt was found to be 0.63% lactic acid which differed significantly ($p<0.01$) with the acidity of dates yoghurt, 0.79% lactic acid. This could be because the addition of dates extract, which contains simple carbohydrates like glucose and fructose, activated the metabolic activity of the added culture (Amerinasab et al. 2015)

Total phenol content and anti-oxidant activity

Dates contain considerable amount of phenolic compounds including flavonoid glycosides, anthocyanins, flavanols, and proanthocyanidins etc which are proved to exhibit act very active antioxidant properties (Ghnimi et al. 2017, Vayalil 2012, Mansouri et al. 2005). According to various studies (Ismail, 2021; Matloob and Balakit, 2016; Vayalil 2012), the total phenolic content of different varieties of dates ranged from 19.88 to 475 mg GAE/100 g, The dates utilised in the current study had a total phenolic content of 198 mg GAE/100 g which was in agreement to the previous findings. Also, the total phenolic content of the optimised dates yoghurt was found to be 59.23mg GAE/100g which indicates that much of the phenolic content in the dates has been passed on to the yoghurt incorporated with it. Similarly, the yoghurt added with dates liquid sugar had phenolic content and was shown to have 0.03µg GAE/mg of yoghurt (Amerinasab et al. 2015).

In terms of antioxidant activity, a strong link was discovered between phenolic content and antiradical efficiency by Mansouri et al. (2005). The fruit extract of dates exhibits a high level of free radical scavenging action and dates extract has proven to be an effective scavenger of reactive oxygen species such as hydroxyl (OH•) and superoxide (O•–) radicals, as well as a potent inhibitor of in vitro macromolecular damages like protein oxidation and lipid peroxidation (Vayalil 2002). The total anti-oxidant capacity of the sample was tested using the DPPH radical (2, 2-diphenyl-1-picryl hydrazyl radical) as per the procedure given by Brand et al. (1995).

The anti-oxidant ability of the sample was measured in terms of IC50 value, i.e, the half maximum inhibitory concentration, which is a measurement of the substance's ability to inhibit a biological or metabolic function. This value specifies how much of a particular component or inhibitor is required to inhibit a biological substance existing in the medium by half. The smaller IC50 value, the higher the radical scavenging rate, hence higher will be the antioxidant capacity. (He et al. 2010). The IC 50 value obtained for the dates yoghurt sample was found to be 19.99mg/100ml. Plain yoghurt tend to have a very lower antioxidant activity. A study conducted by Ye et al. (2013) proved that the antioxidant activity of the plain cow milk yoghurt prepared with 1:1 mixture of *S. Thermophiles and L. bulgaricus* culture exhibited slightly higher IC 50 values (4800mg/100ml) when compared to the yoghurt added with hickory-black soybean yoghurt (4285mg/100ml). On comparison with the IC 50 value obtained for plain yoghurt in the former study, it can be inferred that the addition of dates imparted very high anti-oxidant ability to the dates yoghurt. This high antioxidant activity of the dates yoghurt can be attributed to the phenolic components in dates extract like phenolic acids including ferulic, p coumaric, isoferulic, vanillic acids and flavonoids like quercetin, luteolin, apigenin etc. (Eid et al. 2014), (Pandey and Rizvi 2009)

Color characteristics

The color characteristics of control and dates yoghut are given in Table 4. It can be inferred that the addition of dates extract influences the color parameters of yoghurt ($p<0.01$). The lightness

value (L*) of control and dates yoghurt was found to be 89.02 and 82.04 respectively and hence it can be inferred that the addition of dates extract has reduced the lightness value of yoghurt. The mean values of chromatic components a* and b* of control sample was found to be 0.62 and 17.8 respectively while that of the optimised sample was found to be 2.43 and 14.66 respectively. Here, the a* value is higher for the treated sample which indicates more redness for the sample than the control. The b* value is lower for the treated sample as compared with the control which indicates more yellowness for the control than the sample. Dates consists of color components like melanoidines, and iron polyphenolic complexes which may contribute to the yellow and red colour of dates extract (Fathi et al. 2018). Similar observations were made by Sert et al. (2011) in a study conducted in yoghurt incorporated sunflower and honey into set-type yoghurts.

Conclusions

Dates fruit is renowned for its therapeutic values as it contains many classes of bioactive components such as carotenoids, polyphenols especially phenolic acids isoflavons, lignans, and flavonoids, tannins, and sterols etc. Value addition of yoghurt and other dairy products is gaining much importance in the present scenario. Incorporation of dates extract to yoghurt not only improves the flavour but also enhances the nutritive quality of the product. However, the textural analysis of all the treatments along with the control yoghurt suggested that the textural properties, especially firmness or hardness was deleteriously affected with the increase in levels of dates extract. Meanwhile as the sensory scores obtained was higher for the treatment with 34% dates extract, it was selected as the optimum product for further physicochemical analysis. The total phenolic content in dates yoghurt suggested that a considerable amount of phenolics in dates extract was transferred into the product. It was also proved that the dates yoghurt showed anti-oxidant activity, which can be attributed to the phenolic compounds present in dates like ferulic acid, vanillic acid, coumaric acid etc. Considering the textural quality, yoghurt added with dates extract would find more appropriate use as drinking or stirred type than set type yoghurt. Further studies may be done to scale up the study on this product for its commercialisation.

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