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Effect of Different Mixed Foods with Pomegranates, Raisins, Stingless Bee Honey, Dates and Figs on Antioxidant Properties

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Currently, pomegranates, raisins, stingless bee honey, dates and figs have been used in many products. However, interactions between multiple ingredients in a product may have a synergistic or antagonistic effect, which could improve or reduce functionality. Therefore, the aim of this study is to determine the interaction effect of different mixed foods from pomegranates, raisins, stingless bee honey, dates and figs. The total flavonoid contents and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of 41 samples designed by simplex centroid mixture design was determined before interaction effect and correlation were analyzed. As a result, only eight out of 41 mixed foods shown the synergistic interactions. Moreover, antioxidant properties were correlated ($R^2 = 0.939$) with flavonoid content. In conclusion, the mixed food that shows synergistic interaction may be utilized in the future development of functional food.

Keywords: Antioxidant, Flavonoid content, Interaction effect, Mixed food.

INTRODUCTION

Food plays an important role in every living things. Good food is treated as a requirement that cannot be neglected or viewed lightly for health reasons and the strength of the body depends entirely on the food. Additionally, there is a strong trend in the market to choose natural products and select those with some specific beneficial function for the human body. In this context, the selection of functional ingredients with the required phytochemical content for the intended use is the primary challenge being faced in formulating nutraceutical products [1]. In the food development science and advanced chemical synthetic capacity, food mixtures have become more established and sophisticated and their reach continues to grow. Multiple food uses can target multiple therapeutic benefits [2], which are becoming increasingly popular and the industry's best option. The use of multiple foods with various mechanisms or modes of action will also steer the effect against a single target or disease and manage it better [3]. In conjunction with that matter, pomegranates, raisins, honey, dates and figs have gained attention due to their health benefits [4].

Pomegranates have gained significant attention as functional foods due to the major pomegranate polyphenols, anthocyanins, hydrolyzable tannins and condensed tannins, which are responsible for potential health benefits [5]. Anthocyanin is a pomegranate bioactive compound that has been studied and linked to many aspects of human disease prevention and treatment, such as the anti-inflammatory and cardioprotective activities attributed to their antioxidant properties [6]. Besides pomegranate, raisin also reported had variety of polyphenols that contribute to antioxidant activity [7]. According to Anderson & Waters [8], raisins consumption on a regular basis may lower the risk of diabetes, improves diabetes glycemic control and lowers the risk of atherosclerotic cardiovascular disease.

The stingless bee, also known as lebah kelulut in Malaysia, is a valuable species that has adapted well to tropical environments and emerged as a viable honey alternative. According to Ahmad *et al*. [9], stingless bee honey has antioxidant, anti-inflammatory and anticancer properties, which are related to the total phenolic and flavonoid content of stingless bees [10]. Besides the antioxidant properties, it has also been proven to have antibacterial potential, even against multi-drug resistant bacteria [9,10]. Moreover, dates, are high in antioxidants, which help to prevent the depletion of intrinsic protection against oxidative cell damage and help defense systems in reducing cell damage [11]. Ali [4] also revealed that the dried figs has nutritional, phytochemical, antioxidant and antibacterial activities and presence of polyphenols result in high antioxidant activity, which contributes to its anticancer, antiinflammation, antiacne and antibacterial properties [12].

Previous studies proved that the therapeutic properties of pomegranates, raisins, stingless bee honey, dates and figs are undeniable, since they have been used in the prevention of many types of disease for decades. Consequently, numerous studies have been highlighted on biological systems as bioactive reactions are incredibly complex in multi-herbal or natural-based products [12]. Alternatively, various techniques and methods for determination of synergism and antagonism have been highlighted in previous studies [13,14]. However, there is limited study found on the antioxidant properties of different food mixtures made from pomegranates, raisins, stingless bee honey, dates and raisins. Therefore, this study aims to determine the interaction effect of different mixture formulations on the flavonoid content and antioxidant properties. Furthermore, the corelation between flavonoid content and antioxidant properties was also evaluated.

EXPERIMENTAL

All the raw materials *viz.* dried dates, dried raisins and stingless bee honey, were purchased from Kedai Arab Enterprise (Muar, Malaysia). Meanwhile, fresh pomegranates were purchased from a warehouse at Premium Warehouse, Shah Alam and fresh figs were collected from a local farm at Batu Pahat, Malaysia. All the ingredients were cleaned, dried and kept in airtight polyethylene bags, in a cool and dry place at a temperature below 5 ºC prior to use. The analytical grade chemicals (Merck, Germany) *viz.* 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, potassium acetate, methanol and aluminium chloride.

Experimental design: The simplex centroid mixture design (Design Expert version 11, USA) was employed in this experiment to formulate a mixture from pomegranates, raisins, stingless bee honey, dates and figs. There were no constraints on the design space and all ingredients were assigned the same range of 0-100. As a result, the sum of all proportions of all independent variables in each mixture is 100%. The mixture design formulated 41 mixtures are presented in Table-1.

Sample preparation: Initially, dried food (dates and raisins) was soaked in filtered water in 1:1 ratio then kept at 4 ± 2 ºC for 24 h. The fresh food (pomegranates and figs) was washed, then the pomegranate peel was removed and the figs were used as whole. The juice of pomegranates arils, figs and soaked raisins and dates were collected by juice maker (KEA0236, Alpha, China) then all the juices are then filtered (Whatman No. 1) individually. After that the food juice was mixed according to the mixed food formulation designed by simplex centroid mixture design.

TABLE-1
MIXED FOOD FORMULATION DESIGNED BY SIMPLEX CENTROID MIXTURE DESIGN

Sample code	Food mixture	Food proportion
1	D:R:P:F	25:25:25:25
2	F	100
3	P:F	50:50
4	R:F:H	33.33:33.33:33.33
5	R:P	50:50
6	D:P:H	33.33:33.33:33.33
7	P:F	50:50
8	D	100
9	P	100
10	D:R:P:F:H	20:20:20:20:20
11	D:P:F	33.33:33.33:33.33
12	D:R:P:F:H	10:10:60:10:10
13	D:R	50:50
14	D:R:P	33.33:33.33:33.33
15	D:R:P:F:H	10:60:10:10:10
16	R:H	50:50
17	R:F	50:50
18	D:P:F:H	25:25:25:25
19	R:H	50:50
20	D:R	50:50
21	D:F:H	33.33:33.33:33.33
22	D:R:P:H	25:25:25:25
23	D:P	50:50
24	D:R:F	33.33:33.33:33.33
25	D:R:P:F:H	10:10:10:10:60
26	H	100
27	P:H	50:50
28	D:R:P:F:H	10:10:10:60:10
29	D:H	50:50
30	F:H	50:50
31	D:R:P:F:H	60:10:10:10:10
32	P:F:H	33.33:33.33:33.33
33	R:P:H	33.33:33.33:33.33
34	D:R:H	33.33:33.33:33.33
35	P:H	50:50
36	D:F	50:50
37	D:R:F:H	25:25:25:25
38	R	100
39	R:P:F	33.33:33.33:33.33
40	R:P:F:H	25:25:25:25
41	D:F	50:50

Dates (D), raisins (R), pomegranates (P), stingless bee honey (H) and figs (F)

Interaction effect: The interaction between individuals and mixtures was measured based on total flavonoid content (TFC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of 41 samples. The aluminium colorimetric chloride method was used to determine the flavonoid content of individual foods and mixtures [15] with slight modification. Then, 1 mL sample solution (0.4 mg/mL) was mixed with 3 mL methanol. After that 0.2 mL of 10% $AlCl_3$ and 0.2 mL of potassium acetate (1 M) were added to the mixture. Before absorption measurements, the solution was kept in the dark for 30 min and then the absorbance was measured at 420 nm against a blank sample by an UV spectrophotometer (T60 U, Perkin-Elmer, USA). Total flavonoid content was calculated using a standard quercetin curve as a standard (0, 10, 50, 100 and 200 g/mL) and expressed as quercetin equivalents per mg of sample (μg QE)/mg.

Antioxidant activity: Furthermore, the antioxidant assay, DPPH radical scavenging activity was determined spectrophotometrically [15] with slight modification. Firstly, 0.1 mM of DPPH methanolic solution was prepared by dissolving 39.4 mg of DPPH in 1000 mL methanol. For 30 min, mixture of 2 mL of sample, 4 mL of 0.1 mM DPPH and 2 mL of methanol were incubated. Similarly, the blank solution (4 mL of 0.1 mM DPPH solution and 2 mL of methanol) was also tested. In comparison to blank sample, the absorption was measured using a UV-vis spectrophotometer (T60 U, PerkinElmer, USA) at 517 nm after incubation. The activity of radical scavenging (inhibition%) of the sample and blank was calculated using eqn. 1:

$$\text{Inhibition (\%)} = \frac{\text{AB} - \text{AA}}{\text{AB}} \times 100 \quad (1)$$

where; the absorption of the blank sample is AB and the absorption of the tested sample is AA.

Then, the interaction effect is defined as the difference between the predicted value and the experimental value of the mixtures [13] as in eqn. 2:

(Predicted value) – (Experimental value) = Difference (2)

where predicted value is the calculated value of mixture by taking the value of the individual experimental results in account, whereas the experimental value is the obtained result for the mixture. The positive values indicate synergic interaction potential, negative values indicate antagonistic interaction potential, while the null value indicates an additive effect.

Statistical analysis: All the results presented as mean ± standard deviation. The significant different (p-values ≤ 0.05) between samples was determine by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Then, Turkey honestly significant different (HSD) multiple rank test was applied. The data was processed by IBM SPSS Statistics Version 26 (USA). Furthermore, correlation analyzed by principal component analysis (PCA). The data was processed by XLSTAT version 21 (USA).

RESULTS AND DISCUSSION

As technology progressed and the demand for the natural antioxidants increased, a variety of foods were combined in the expectation that they would provide greater benefits than synthetic drugs [16]. Hence, this study lays the foundation for future food design and formulation based on antioxidant interactions in pomegranate, raisin, date, fig and stingless bee honey mixtures, which could potentially improve the efficacy of functional food as a chemopreventive agent. The total flavonoid content (TFC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of pomegranates, raisins, stingless bee honey, dates and figs were determined. The result shows that pomegranates (17.07 μg QE/mg, 48.05%) has significantly (p < 0.5) highest TFC and DPPH values, followed by raisins (13.97 μg QE/mg, 46.87%), dates (13.20 μg QE/mg, 36.13%), stingless bee honey (12.19 μg QE/mg, 30.78%) and figs (8.06 μg QE/mg, 26.44%) (Fig. 1).

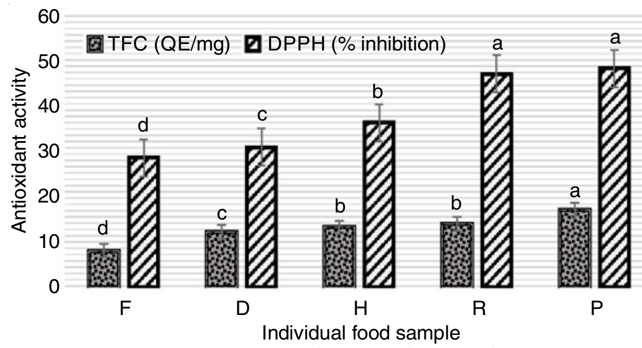

Fig. 1. TFC and DPPH values of quranic food; F (figs), D (dates), H (stingless bee honey), R (raisins), and P (pomegranates)

The DPPH inhibitory percentage of pomegranates in this study was found within the range 31.16% to 66.82% as reported by Malek *et al.* [1] but higher than the range value revealed by Wu & Tian [6], which is 15.59% to 40.72%. Previous study reported that stingless bee honey DPPH inhibitory percentage value in the range of 12.34-29.52% [9] and 53.33-97.30% [10]. However, this study finding contradict with both reports. Besides that, the result showed that DPPH inhibitory percentage of dates is lower as compared to 96.3% as reported by Assadi *et al.* [11]. However, for figs, the DPPH inhibitory percentage value is concurrent with the range of 8.96 to 88.91% as reported by Mahmoudi *et al.* [12]. The different flavonoid content and DPPH inhibitory percentage values might be due to different extraction methods applied [17]. This study only utilized water extraction method while others study utilizes different solvent extraction method.

The interaction effect determined by the differences of experimental and predicted where positive values indicate the potential of synergistic effect, while negative value indicate the potential of antagonistic effect. Subsequently, Table-2 shows the result of interaction effect of 41 mixtures studies. The TFC values range between 1.26 μg QE/mg (sample 32) and 22.37 μg QE/mg (sample 5) while the DPPH inhibition range is 9.56% (sample 4) to 49.68% (sample code 5), which is lower than individual raisin and pomegranate but higher than honey, date and fig. From the interaction effect results, only eight out of 41 mixtures show synergistic interaction in TFC and DPPH. The combination of raisin and pomegranate (sample 5) in the same ratio has shown significantly (p < 0.5) highest percentage inhibition of DPPH (49.68%), which is higher than pomegranate and raisin individually. Meanwhile, the combination of raisins, figs and stingless bee honey (sample 4) shows lowest antioxidant activity (9.56%) compared to others mixture.

There is limited study found on the mixture of figs, dates pomegranates, raisins and stingless bee honey. Amin *et al*. [3] reported that the combination of pomegranates and grapes show lower antioxidant activity when compared to pomegranates alone. Another findings has been also reported by Assadi *et al.* [11] on pomegranate and date mixture, which revealed that the mixtures had lower antioxidants than pomegranate and date individually. Those previous findings were contradicting with this study finding. This might be because of different processing, extraction and storage conditions resulting in degradation of antioxidants, which also supported by Yang *et al*. [17].

TABLE-2
FOOD-FOOD INTERACTION IN TFC AND DPPH SCAVENGING ACTIVITY

Sample code	TFC		Interaction effect	DPPH		Interaction effect
	Predicted value	Experimental value		Predicted value	Experimental value	
Single food						
2	NA	8.06	NA	NA	26.44	NA
8	NA	12.19	NA	NA	30.78	NA
9	NA	17.07	NA	NA	48.05	NA
26	NA	13.20	NA	NA	36.13	NA
38	NA	13.97	NA	NA	46.87	NA
Mixture of two foods						
13	13.59	16.70^{bcd}	Synergistic	38.83	40.28^{bc}	Synergistic
29	12.70	14.63^{cdef}	Synergistic	33.46	35.49^{def}	Synergistic
5	15.52	22.37^{a}	Synergistic	47.46	49.68^{a}	Synergistic
7	12.57	13.07^{cdefgh}	Synergistic	38.25	40.59^{hijk}	Synergistic
3	12.57	13.07^{cdefgh}	Antagonistic	38.25	30.59^{hijk}	Antagonistic
16	13.09	8.52^{hijkl}	Antagonistic	41.50	23.07^{mn}	Antagonistic
17	11.02	9.46^{ghijk}	Antagonistic	37.66	22.68^{mn}	Antagonistic
19	13.09	8.52^{hijkl}	Antagonistic	41.50	23.07^{mn}	Antagonistic
23	15.14	11.17^{efghi}	Antagonistic	39.42	40.28^{bc}	Antagonistic
27	14.64	13.07^{cdefgh}	Indifferent	42.09	25.77^{lm}	Antagonistic
30	10.13	2.04^{mn}	Antagonistic	32.29	17.98^{op}	Antagonistic
35	14.64	13.07^{cdefgh}	Antagonistic	42.09	25.77^{lm}	Antagonistic
41	10.63	6.10^{klm}	Antagonistic	42.09	25.77^{lm}	Antagonistic
Mixture of three foods						
24	11.74	12.6^{cdefghi}	Synergistic	35.36	38.01^{ghi}	Synergistic
11	12.78	16.63^{bcd}	Synergistic	35.75	37.88^{no}	Synergistic
4	11.41	4.86^{lmn}	Antagonistic	13.03	9.56^{r}	Antagonistic
6	14.16	9.46^{ghijk}	Antagonistic	38.32	14.54^{pqr}	Antagonistic
14	14.75	9.46^{ghijk}	Antagonistic	41.90	35.25^{def}	Antagonistic
21	11.15	11.09^{efghi}	Antagonistic	31.78	12.93^{qr}	Antagonistic
32	14.97	1.26^{n}	Antagonistic	28.37	14.55^{pqr}	Antagonistic
33	14.41	13.63^{ab}	Antagonistic	43.68	34.34^{efg}	Antagonistic
34	13.12	11.73^{efghi}	Antagonistic	37.92	36.61^{def}	Antagonistic
39	13.03	9.56^{ghijk}	Antagonistic	41.12	16.55^{pq}	Antagonistic
Mixture of four foods						
18	12.63	14.93^{cdef}	Synergistic	35.85	36.52^{fgh}	Synergistic
1	13.08	11.03^{efghij}	Antagonistic	38.54	36.06^{def}	Antagonistic
22	14.11	11.67^{efghi}	Antagonistic	40.46	42.83^{b}	Antagonistic
37	11.54	8.51^{hijkl}	Antagonistic	33.75	29.77^{ijk}	Antagonistic
40	12.825	11.60^{efghi}	Antagonistic	39.87	26.64^{l}	Antagonistic
Mixture of five foods						
31	13.05	15.60^{bcde}	Synergistic	34.42	35.16^{hij}	Synergistic
10	12.90	11.09^{efghi}	Antagonistic	38.05	36.54^{def}	Antagonistic
12	14.99	12.00^{efghi}	Antagonistic	43.05	37.76^{cd}	Antagonistic
15	13.44	11.93^{efghi}	Antagonistic	42.46	36.76^{de}	Antagonistic
25	12.55	4.80^{lmn}	Antagonistic	37.09	21.71^{ghijk}	Antagonistic
28	10.48	10.37^{fghijk}	Antagonistic	33.25	25.77^{lm}	Antagonistic

Aside from the synergistic effect of four mixtures of two foods (samples 5, 7, 13 and 29), only two mixtures of three foods (samples 11 and 24), one mixture of four foods (sample 18) and one mixture of five foods (sample 31) had a synergistic effect. It is observed that the more food added, the more antagonistic the effect. This could be due to the interactions between flavonoids, which can increase or decrease the availability of hydroxyl groups, affecting antioxidant properties [13]. This finding supported by Eghbaliferiz & Iranshahi [18], which revealed that multicomponent combinations have the ability to change chemical properties and affecting biological activity. Furthermore, Farooq & Sehgal [2] also reported that interaction between bioactive components from various foods can affect the therapeutic potential either synergistically or antagonistically.

Flavonoids are known to be the phytochemicals in foods that contribute to the curative properties such as the prevention of cancer or any other ailment due to their high antioxidant properties as mentioned in a previous study [16]. Fig. 2 shows that correlation between TFC and DPPH scavenging activity with the value of $R^2 = 0.939$. This result explains the trend of highest TFC shows higher in DPPH. This finding agreed with Ma & Huang [19], which stated that antioxidant activity was significantly correlated with TFC. In another study, Khemakhem

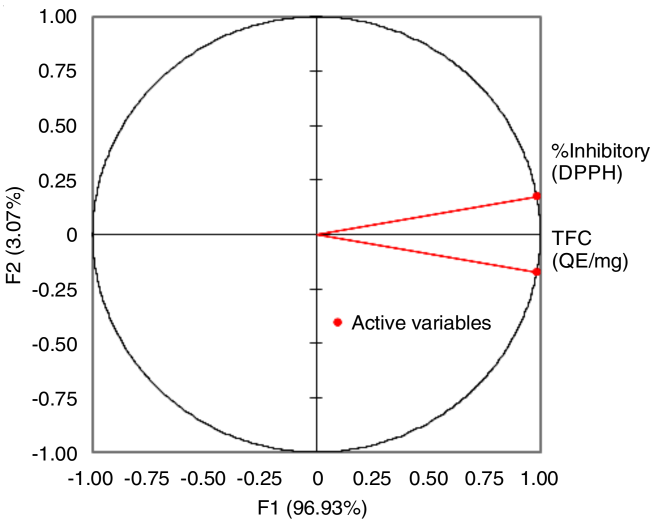

Fig. 2. Correlation between TFC and DPPH scavenging activity

et al. [20] reported that natural antioxidants are primarily related to their phenolic and flavonoid content. Moreover, Keng *et al.* [10] reported that flavonoids registered more hydrogen donation than phenolic acids and were twice as effective as vitamin E in radical scavenging.

Conclusion

It is emphasized that the effectiveness of pomegranates, dates, raisins, figs and honey is based on the presence of multiple phenolic and flavonoid compounds, which contribute to their therapeutic properties. It can be concluded that pomegranates, dates, raisins, figs and honey contained abundant quantity of polyphenols especially flavonoids and their combination cause an interaction of polyphenols. As a result, the possibility of interaction with the radical DPPH can be increased or decreased. Moreover, the appropriate proportion of a food mixture may contribute to a synergistic interaction while inappropriate proportion results in an antagonistic interaction. Therefore, it is important to optimize the formulation of food combined to ensure the combination of food would provide greater benefits than synthetic drugs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. S.N.A.A. Malek, H. Haron, W.A.W. Mustafa and S. Shahar, *J. Agric. Sci.*, **9**, 50 (2017); https://doi.org/10.5539/jas.v9n13p50
2. S. Farooq and A. Sehgal, *J. Food Biochem.*, **43**, e12984 (2019); https://doi.org/10.1111/jfbc.12984
3. C.E. Childs, P.C. Calder and E.A. Miles, *Nutrients*, **11**, 1933 (2019); https://doi.org/10.3390/nu11081933
4. M. Ali, *Afr. J. History and Culture*, **6**, 141 (2014); https://doi.org/10.5897/AJHC2014.0188
5. P. Kandylis and E. Kokkinomagoulos, *Foods*, **9**, 122 (2020); https://doi.org/10.3390/foods9020122
6. S. Wu and L. Tian, *Molecules*, **22**, 1606 (2017); https://doi.org/10.3390/molecules22101606
7. A.B. Mnari, A. Harzallah, Z. Amri, S.D. Aguir and M. Hammami, *Int. J. Food Prop.*, **19**, 578 (2016); https://doi.org/10.1080/10942912.2015.1038720
8. J.W. Anderson and A.R. Waters, *J. Food Sci.*, **78(Suppl.1)**, A11 (2013); https://doi.org/10.1111/1750-3841.12071
9. F. Ahmad, P. Seerangan, M.Z. Mustafa, Z.F. Osman, J.M. Abdullah and Z. Idris, *Malays. J. Med. Sci.*, **26**, 30 (2019); https://doi.org/10.21315/mjms2019.26.2.4
10. C.B. Keng, H. Haron, R.A. Talib and P. Subramaniam, *J. Agric. Sci.*, **9**, 32 (2017); https://doi.org/10.5539/jas.v9n13p32
11. I. Assadi, W. Elfalleh, M. Benabderrahim, H. Hannachi, W. Chaalen and A. Ferchichi, *Int. J. Fruit Sci.*, **19**, 300 (2019); https://doi.org/10.1080/15538362.2018.1512438
12. S. Mahmoudi, M. Khali, A. Benkhaled, I. Boucetta, Y. Dahmani, Z. Attallah and S. Belbraouet, *Eur. J. Hortic. Sci.*, **83**, 104 (2018); https://doi.org/10.17660/eJHS.2018/83.2.6
13. M. Hidalgo, C. Sánchez-Moreno and S. de Pascual-Teresa, *Food Chem.*, **121**, 691 (2010); https://doi.org/10.1016/j.foodchem.2009.12.097
14. E. Kurin, P. Mucaji and M. Nagy, *Molecules*, **17**, 14336 (2012); https://doi.org/10.3390/molecules171214336
15. A. Pekal and K. Pyrzynska, *Food Anal. Methods*, **7**, 1776 (2014); https://doi.org/10.1007/s12161-014-9814-x
16. D.J. Newman and G.M. Cragg, *J. Nat. Prod.*, **83**, 770 (2020); https://doi.org/10.1021/acs.jnatprod.9b01285
17. C. Yang, X. Jiang, L. Ma, W. Xiong, S. Zhang, J. Zhang and L. Zhang, *J. Food Sci.*, **86**, 1751 (2021); https://doi.org/10.1111/1750-3841.15682
18. S. Eghbaliferiz and M. Iranshahi, *Phytother. Res.*, **30**, 1379 (2016); https://doi.org/10.1002/ptr.5643
19. Y. Ma and H. Huang, *Int. J. Food Sci. Technol.*, **49**, 2290 (2014); https://doi.org/10.1111/ijfs.12545
20. M. Khemakhem, Y. Zarroug, K. Jabou, S. Selmi and N. Bouzouita, *J. Food Sci.*, **86**, 852 (2021); https://doi.org/10.1111/1750-3841.15636