Asian Journal of Chemistry; Vol. 36, No. 1 (2024), 255-262

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2024.30698

Biological Activity and Pectin Content in Orange, Lemon, Tangerine, Grapefruit and Kumquat Peels in Vietnam

B.N. HOANG[1], T.C.Q. NGO[1], T.H.N. TRAN[2], D.N. DANG[2], T.T. TRAN[1], Q.K.A. NGUYEN[1], Q.C. NGUYEN[1] and N.H.K. NGUYEN[1,3]

[1]Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
[2]Faculty of Chemical Engineering and Food Technology, Nong Lam University, Ho Chi Minh City, Vietnam
[3]College of Agriculture, Can Tho University, Can Tho City, Vietnam

*Corresponding author: E-mail: bichhn@ntt.edu.vn

Received: 10 October 2023; *Accepted*: 27 December 2023; *Published online*: 31 December 2023; AJC-21511

Extracts of orange, lemon, tangerine, pomelos and kumquat peels were extracted by two methods *viz*. solvent extraction and ultrasound-assisted extraction. Extracts from peel were evaluated for their moisture, biological activity and antibacterial capacity. Raw material powder after preliminary processing tends to turn golden brown with moisture content below 10%. The antioxidant capacity of the extract from low to high (orange > tangerine > pomelos > lemon > kumquat) was found. The vitamin C equivalent of tangerine is the highest recorded at the value of 1.30 mg g^{-1}. In addition, the total polyphenol and flavonoid content of pomelos and tangerine was found to be higher than that of other extracts. The antibacterial ability was also evaluated based on four intestinal-specific strains including *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. aureus*. The orange peel extract isolated from solvent extraction method has the highest resistant to bacterial strains, whereas, the other extracts have moderate antibacterial activity. The results on the pectin composition in the peels have also been specifically evaluated. All the types of pectin powders have a degree of esterification (DE) coefficient < 50% corresponding to pectin LM (low-methoxyl), especially pectin from lemon peel gives a DE coefficient > 50% corresponding to pectin HM (high-methoxyl).

Keywords: Biological activity, Pectin components, Rutaceae family, Citrus peel.

INTRODUCTION

The citrus family has the scientific name of Rutaceae with 6 subfamilies, 154 genera and more than 2119 species of plants [1]. It is widely distributed worldwide and concentrated mainly in tropical and temperate zone [2]. According to the Food and Agriculture Organization (FAO), the world supply of citrus fruits in 2019 reached 157.98 million tons, including oranges (48.8%), tangerines (22.4%), pomelos (5.8%), lemon and lime (12.7%) and the other citrus fruits (9.2%). The applications of citrus in food technology was developed 100 years ago [3]. Citrus genus is not only applied in food technology but also in many other fields [3]. Although there have been many practical applications, the impact of citrus on the environment is quite large. As reported by Panwar *et al*. [4], the byproducts after processing are peel (50-70%), pulp (60-65%), seeds (30-35%) and residue (less than 10%). Citrus byproducts contain about 80-90% moisture and large amounts of organic matter [4,5]. Organic compounds in citrus byproducts have high content including sugars, carbohydrates, proteins, organic acids, lipids, essential oils, pigments like carotenes, vitamins and polyphenols [4,6,7]. Therefore, to take advantage of this byproduct, researchers have used shells to prepare essential oils, waste residues are used to compost as organic fertilizers.

Essential oils from citrus fruits have been commercialized for a long time and are often used in the cosmetic and environmental industries. However, the potential applications of citrus byproducts is quite limited. Therefore, the researchers studied extracts from the peels and seeds of citrus trees with polyphenol compounds such as hesperidin, eriocitrin [8], neohesperidin [9], vanillin [10], limonene, neral, *trans*-verbenol, narirutin, naringin, sinensetin, tangeretin, nobiletin, caffeic, *p*-coumaric, ferulic, decanal and sinapic acids [11,12]. There are also a number of organic compounds such as flavanoinic, vitamin C

[13], folic acid, potassium and pectin [14]. Organic compounds in citrus peels have been studied for their anticancer potential such as nobiletin [15] and flavonoid compounds [16].

After the process of solvent extraction or extracting essential oils, the rind residue of citus still contains pectin present in the peel. Pectin is a known natural polymer compound and is extracted from the citrus genus. In 2021, Hu *et al*. [17] extracted pectin from citrus byproducts including lemon (21.76%), pomelos (20.17%) and orange (20.81%), at a rate greater than that of commercial pectin (17.10%). Recently, Picot-Allain [18] investigated the antioxidant capacity of pectin in orange and lemon peels and their ability to inhibit cholesterol esterase in the pancreas. Acid hydrolysis is the most widely used method for pectin extraction, which has the advantages of low cost, easy operation and environmental protection [19]. Citrus peels have a high potential for use in medicines, foods and cosmetics due to their essential oil content, natural organic compounds and pectin components. To understand in depth about the benefits of bioactive chemical compounds and pectin components obtained from various kinds citrus peels *e.g*. lemon, orange, grapefruit, kumquat and tangerine skins in Vietnam is discussed. Different types of citrus peels were purchased from food processing facilities and extracted organic compounds such as polyphenols, flavonoids, *etc*. and evaluated for their antioxidant capacity. The residue obtained after extracting the extract will be further processed to extract the pectin component present in the peel and then the ratio of pectin components will be established using pectin powder.

EXPERIMENTAL

Sodium hydroxide, citric acid, sodium carbonate anhydrous, aluminium chloride, hydrochloric acid (37%) and ethanol (≥ 95% purity) were supplied from Xilong Scientific Co., Ltd. China. The Folin-Ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid (vitamin c), gallic acid, phenolphthalein reagent and quercetin standard were procured from Sigma, USA. After collecting the citrus peel samples, the damaged parts were discarded and washed several times with water to remove dirt. Samples were dried at 70 ºC for 48 h and finely ground into powder.

Solvent extraction: The solvent based extraction process was carried out by following the method suggested by To & Muoi [20] with some modifications. The citrus peel powder (1 g) was added to 15 mL of 70% ethanol and soaked for 24 h at room temperature. The extract was recovered after filtration to remove the residue. The solution was stored in the sample tube at 10 ± 5 ºC in the refrigerator.

Ultrasound-assisted extraction: The extraction process was carried out by adopting the method as reported by Esteve *et al.* [21] with some modifications. The citrus peel powder (1 g) was added to 15 mL of 70% ethanol and ultrasonic for 60 min at room temperature. The extract was recovered after filtration to remove the residue. The solution was stored in the sample tube at 10 ± 5 ºC in the refrigerator.

Preparation of pectin: Weighed 1 g citrus peel powder, add ed15 mL citric acid solution (1 M) and stirred at 70 ºC for 120 min, speed 500 rpm and pH 1.5. The solution was washed with ethanol and centrifuged at 6000 rpm for 15 min to obtain a solid. Samples will be washed several times to pH 4-5 and dried at 60 ºC for 24 h to obtain pectin powder.

Colourimetric method: The colour system L* a* b* (also known as CIELAB) was determined using a CR-400 Konica Minolta colorimeter, Japan. L* signifies the lightness of colour, ranging from black (0) to white (+100). The value of a* is the trend in the colour of a surface, ranging between green (-100) and red (+100). The value of b* represents the trend in the colour of a surface ranging between blue (-100) and yellow (+100) [22].

Moisture (%): The samples were dried at 105 ºC to constant weight by adopting the standard AOAC 934.06 method.

DPPH scavenging analysis: The *in vitro* antioxidant activity was assessed by following DPPH free radical scavenger method using an ascorbic acid standard [23]. Briefly, 1 mL of ethanol DPPH solution (0.6 mM) was thoroughly mixed with 1 mL of concentrated extract (10-60 mg mL^{-1}) and incubated in dark for 60 min. The absorbance will then be recorded at 517 nm in a UV-VIS spectrophotometer and the inhibition concentration at 50% value (IC_{50}) will be calculated from the ascorbic acid calibration curve [24]. The DPPH* free radical scavenging activity (OR %) was determined based on the formula. The results were recorded based on the OR% value at the specified dilution concentration:

$$\text{Free radical scavenging activity (\%)} = \frac{Abs_C - Abs_T}{Abs_C} \times 100$$

where Abs_C: Optical absorbance of the control sample and Abs_T: Optical absorbance of the specimen.

Determination of total phenolic content (TPC): The total phenolic content (TPC) was determined by the Folin-Ciocalteu method by following Pham *et al.* [25] method with some modifications. In brief, 0.5 mL of extract or standard gallic acid solution (with concentration from 0.05 to 3 mg mL^{-1}) was added to 2.5 mL of Folin-Ciocalteu reagent (1:10) and mixed well. After 4 min, 2 mL of saturated Na_2CO_3 solution was added, shaked and then incubated for 2 h at room temperature. The absorbance of the solution after the reaction was measured at 760 nm. Gallic acid was used as a reference standard and the results are expressed as milligrams of gallic acid per gram of sample material.

Determination of total flavonoid content: The total flavonoid content (TFC) was also determined according to the method of Hung *et al.* [25] with some modifications. About 0.5 mL of sample solution (diluted to appropriate concentration) was added to 0.1 mL of 10% $AlCl_3$ solution followed by the addition of 0.1 mL of CH_3COOK solution (1 M) and 4.3 mL of distilled water and finally mixed thoroughly. Leave the solution at room temperature for 30 min. Then measure the optical absorbance at 415 nm on a UV-Vis spectrophotometer. Quercetin was used as standard and the total flavonoid content is expressed as a percentage of the milligram equivalent of quercetin in 1 g of dry matter compared with the sample.

Antimicrobial activity: The antimicrobial activity experiments were conducted by following the method of Khane *et al.* [26] with some modification, In brief, 100 µL of bacterial

suspension was poured on the medium dish containing evenly spreaded agar. With the help of a sterile cork drill with a diameter of 7 mm to create perforations in the agar and then injected 70 µL of distilled water for the negative control well, 70 µL of ampicillin as a positive control and 70 µL of extract into the marked holes. The plate was then incubated at 37 ºC for 24 h.

Degree of esterification (DE): A pectin powder (5 g) was mixed with 10 mL distilled water, 5 mL ethanol, 1 g NaCl followed by the addition of few drops of phenolphthalein in a conical flask and finally shaked thoroughly and diluted the solution with distilled water till the volume become 100 mL. Titrate the solution with 0.1 N NaOH until the colour turns pink, then hold steady for 30 s and record the results. Then esterify the solution by adding 25 mL of 0.25 N NaOH solution with continuous stirring and allowed to stand for 30 min. Neutralized the solution with 25 mL of 0.25 N HCl. Finally, the mixture was titrated again with 0.1 N NaOH solution. The results were recorded and calculated:

$$DE\ (\%) = \frac{V_2}{V_2 + V_1} \times 100$$

where DE: degree of esterification of pectin, V_2: volume of 0.1 N NaOH solution titrated for second time (mL), V_1: volume of 0.1 N NaOH solution titrated for first time (mL).

Methoxyl index (MI): Determination of methoxyl index was investigated according to the method of Dhushane & Mahendran [27]. Placed 0.5 g of pectin into a 250 mL Erlenmeyer flask, moistened with 5 mL of ethanol and then added 1 g of NaCl and 100 mL of distilled water (make sure the pectin is completely dissolved and free of lumps). Then, added 6 drops of phenol red as indicator and titrated with 0.1 N NaOH until a pink colour appears. The mixture was added 25 mL of NaOH solution (0.25 N), shaked thoroughly and allowed to stand for 30 min. Then, 25 mL of 0.25 N HCl was added to the above mixture and titrated again with 0.1 N NaOH solution and V_{NaOH} was obtained. The methoxyl index (MI) was calculated according to the formula:

$$MI\ (\%) = \frac{V_{NaOH} \times CN_{NaOH} \times 3.1}{m}$$

where V_{NaOH}: volume of final titration solution (mL), CN_{NaOH}: concentration of titrated NaOH solution (N), m: mass of raw pectin sample (g).

Pectin yield (%): The extraction efficiency of pectin (%) was calculated according to the following formula:

$$\text{Pectin yield}\ (\%) = \frac{m_2}{m_1} \times 100$$

where m_1: weight of the starting material (g), m_2: volume of pectin powder obtained after extraction (g).

Determination of pectin (P) composition: Weighed 10 g of sample was added to 100 mL of 0.1 N NaOH in an Erlenmeyer flask and mixed well. To mke saponify the pectin, leave the mixture stirred overnight. Filtered the mixture through filter paper and added 50 mL 1 N CH_3COOH to the filtrate. After 5 min, added 50 mL $CaCl_2$ (2.0 N) and left for 1 h. Now boiled the mixture for 5 min, then filtered through a filter paper to obtain the constant mass and weight (m_1). Washed the calcium pectate precipitate with hot distilled water until there are no Cl^- ions (test the wash water with 1% AgCl solution until no white precipitate is present). Placed the filter paper with the precipitate in the oven, dry to constant weight and weigh (m_2):

$$P\ (\%) = \frac{(m_2 - m_1) \times 0.92}{m}$$

where m: weight of sample (g), m_1: weight of filter paper (g), m_2: mass of filter paper and calcium pectate precipitate (g), 0.92: conversion factor from calcium pectate to pectin (*i.e.* pectin makes up 92% by mass of calcium pectate).

RESULTS AND DISCUSSION

Antioxidant activity: Extracts of orange, lemon, tangerine, grapefruit and kumquat peels were extracted by two methods: solvent extraction (SE) and ultrasound-assisted extraction (UE) methods. The antioxidant capacity was determined by IC_{50} value, mg vitamin C per g dry weight (mg g^{-1}) and shown in Table-1. The IC_{50} value was calculated by regression equation based on the evaluation of DPPH by concentration range [26]. For DPPH method, the lower IC_{50} value is the higher antioxidant capacity [28]. The IC_{50} value of vitamin C (ViC) was recorded at a concentration of 0.005 mg mL^{-1}. The results showed

TABLE-1
IC_{50} VALUES AND VITAMIN C EQUIVALENTS OF EXTRACTS FROM ORANGE, LEMON, TANGERINE, GRAPEFRUIT AND KUMQUAT PEELS BY DPPH METHOD

Method	Sample	Regression equation	R^2	IC_{50} (mg mL^{-1})	Equivalent vitamin C/ dry weight (mg g^{-1})
	Vitamin C	y = 16.47ln(x) + 139.12	0.95	0.005	–
Ultrasound-assisted extraction	Grapefruit	y = 26.155ln(x) + 4.534	0.89	8.05	1.28 ± 0.10
	Lemon	y = 33.974ln(x) – 25.639	0.96	9.27	1.23 ± 0.09
	Orange	y = 32.989ln(x) – 24.309	0.99	9.51	1.13 ± 0.06
	Tangerine	y = 27.905ln(x) – 2.1638	0.86	6.48	1.34 ± 0.13
	Kumquat	y = 25.541ln(x) – 8.7762	0.98	9.99	0.94 ± 0.03
Solvent extraction	Grapefruit	y = 19.27ln(x) + 24.588	0.95	3.74	1.29 ± 0.11
	Lemon	y = 16.548ln(x) + 30.541	0.88	3.24	1.20 ± 0.10
	Orange	y = 18.554ln(x) + 19.179	0.94	5.26	1.15 ± 0.10
	Tangerine	y = 20.331ln(x) + 19.022	0.90	4.59	1.30 ± 0.12
	Kumquat	y = 11.887ln(x) + 41.644	0.85	2.02	1.16 ± 0.10

that the tangerine sample yield the lowest value of 6.48 mg mL^{-1}. For SE method, the kumquat sample gave the lowest value of 2.02 mg mL^{-1}. The IC_{50} values of SE method are twice or three times lower than those of UE method. This shows that organic compounds extracted from solvent have better oxidation resistance than ultrasonic. Although the vitamin C equivalent concentrations from both methods were similar and have no significant difference. Equivalent ViC/dry weight results showed that tangerine sample had the highest concentrations of 1.34 mg g^{-1} (UE) and 1.3 mg g^{-1} (SE). This shows that biologically active substances with antioxidant capacity such as vitamin C in the sample account for only about 1.0-1.5 mg. This result is consistent with the concentration of 0.93 mg g^{-1} obtained by Esteve *et al.* [21] for vitamin C equivalent concentrations in orange peel extract by UE method. Lin *et al.* [29] also showed that the IC_{50} value of pomelos peel ranged from 1 to 4 mg mL^{-1} by SE method. The results show agreement with other studies with the SE and UE methods. Comparing the two methods, SE method gives better results than UE method.

Total flavonoid content: The evaluation results of extracts from oranges, lemons, tangerines, pomelos and kumquats peels are show in Fig. 1. The flavonoid contents of grapefruit, oranges, tangerines, lemons and kumquats by SE and UE methods were 4.22, 4.24, 4.18, 1.43 and 3.54 mg g^{-1}; and 3.57, 2.50, 3.42 , 3.20 and 4.22 mg g^{-1} respectively. These results show that the flavonoid content of the SE method is higher UE method for samples of grapefruit, oranges and tangerines and *vice-versa* for samples of lemons and kumquats.The study of Lin *et al.* [29] also showed that the TFC of pomelos was 3.8 mg g^{-1}. Another study conducted by Anticona *et al.* [30], the TFC of oranges was also evaluated at 2.7 mg g^{-1}. It can be seen that the observed TFC content is equivalent to the published studies. Between the two methods, the SE method showed that TFC content was higher than that of the UE method. This may be due to the extraction time to release the flavonoid compounds present in the peel powder.

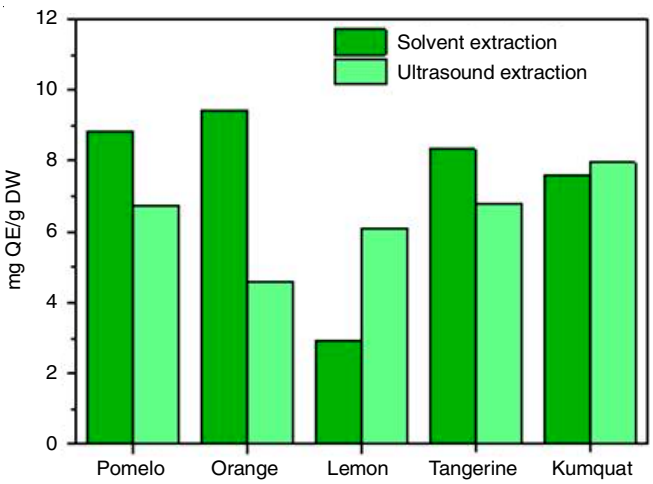

Fig. 1. Total flavonoid content of orange, lemon, tangerine, grapefruit and kumquats peel extracts from SE and UE methods

Total polyphenol content: The phenolic content of any plant is directly related to their antioxidant properties. Phenolic compounds act as reducing agents, hydrogen donors and are capable of scavenging free radicals [31]. The presence of a significant amount of phenol can significantly contribute to the antioxidant properties. The evaluation results of polyphenol content extracted from grapefruit, orange, lemon, tangerine and kumquat peels are shown in Fig. 2. The polyphenol content of grapefruit, oranges, tangerines, lemons and kumquats by SE and UE methods were 18.99, 15.78, 18.07, 11.52, 15.41 mg g^{-1} and 15.46, 11.54, 15.41, 12.42 and 13.07 mg g^{-1}. Anticona *et al.* [30] evaluated the polyphenol content in oranges, lemons and grapefruits at 15 mg g^{-1}, 18 mg g^{-1} and 12 mg g^{-1}, respectively. Whereas Hung *et al.* [25] reported the polyphenol contents extracted by UE method was 4.9 mg g^{-1} for green-skinned pomelo, 4 mg g^{-1} for Nam Roi pomelo and 6.8 mg g^{-1} for Tan Trieu pomelo [25]. Similalry Suri *et al.* [32], the polyphenol content of sweet lime was determined to be 25 mg g^{-1}. It can be observed thatin all the reported cases, the polyphenol content is still between 11 and 19 mg g^{-1} which is consistent with published studies. These results showed that the polyphenol content from the two extraction methods is nearly the same. This shows that using the UE method has the potential to replace the SE method, saving time and operating costs.

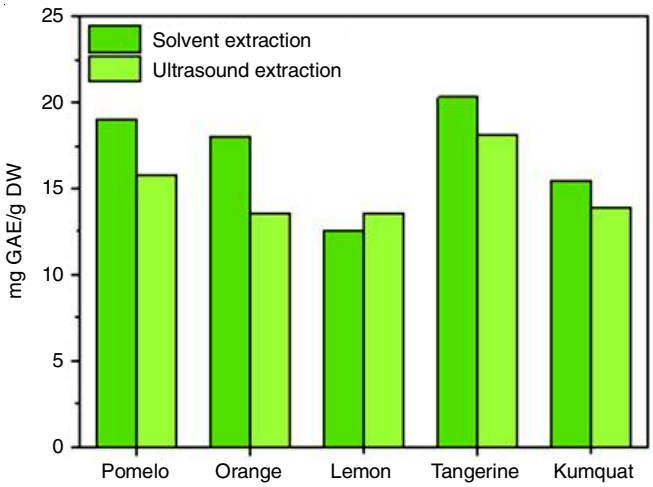

Fig. 2. Total polyphenol content of orange, lemon, tangerine, grapefruit, kumquats peel extracts from SE and UE methods

Antimicrobial activity: The antibacterial ability was evaluated against four microorganisms including *Escherichia coli*, *Bacillus subtilis, Pseudomonas aeruginosa*, *Staphylococcus aureus*. The inhibition zone diameters (mm) of orange, lemon, tangerine, grapefruit and kumquats peel extracts from SE and UE methods are shown in Table-2. For *E. coli* strain, only 0.9 mm was recorded inhibition zone diametersof the kumquats sample by the UE method. There is also an SE method oranges sample recorded at 2.2 mm. For strain *B. subtilis*, Inhibition zone diameters were recorded for samples from SE method. In addition, pomelos samples by UE method were recorded 0.7 mm. For strain *P. aeruginosa*, no value was recorded other than the pomelos extract sample. Orange and kumquat samples from the two methods were recorded as 0.9 mm and 1.1 mm for the UE method, 1.9 mm and 1.8 mm for the SE method, respectively. For *S. aureus* strain, all samples have inhibition

TABLE-2
ANTIMICROBIAL ACTIVITIES OF ORANGE, LEMON, TANGERINE, POMELOS, KUMQUATS PEEL EXTRACTS FROM SOLVENT EXTRACTION (SE) AND ULTRASOUND-ASSISTED EXTRACTION (UE) METHODS

Method	Sample	Inhibition zone diameters (mm)			
		E. coli	*B. subtilis*	*P. aeruginosa*	*S. aureus*
Ultrasound-assisted extraction	Pomelos	–	0.7	–	0.9
	Lemon	–	–	–	1.1
	Orange	–	–	0.9	0.8
	Tangerine	–	–	–	0.7
	Kumquat	0.9	–	1.1	2.2
Solvent extraction	Pomelos	–	2.9	–	1.9
	Lemon	–	2.8	1.6	1.2
	Orange	2.2	0.9	1.9	1.6
	Tangerine	–	1.6	0.9	2.1
	Kumquat	–	0.9	1.8	1.7

zone diameters. The results showed that the extract had antibacterial ability against strong to weak bacterial strains (*S. aureus* > *P. aeruginosa* > *B. subtilis* > *E. coli*). Looking at the two methods, it can also be seen that the SE method gives better antibacterial ability than the UE method.

Yield, colourimetry and moisture of pectin: Pectin powder from peels of oranges, lemons, tangerines, grapefruits and kumquats after extraction was evaluated for moisture content and CIELAB colour space. The yield and moisture content of the samples are shown in Table-3. It can be seen that the pectin extraction efficiency of lemon and kumquat samples (46% and 41.8%) is higher than that of oranges, grapefruits, tangerines (37, 38.5 and 39%). It can be seen that the extraction efficiency of pectin powder is quite high and almost 50%. In which, the moisture content of the sample was also recorded as 1.78% (orange), 1.3% (lemon), 8.2% (tangerine), 3% (grapefruit), 1.61% (kumquats).The results showed that the moisture content of pomelo and tangerine samples was the highest but to the lowest yield. High extraction efficiency shows potential in the practical application. For a comprehensive assessment, the colour powder and characterization of pectin components are also focused. The colour of pectin powder is assessed using The Commission Internationale de l'éclairage Lab colour space value (CIELAB colour space). The samples evaluated were pectin powder of oranges, lemons, tangerines, grapefruits, kumquats. The CIELAB colour space of pomelos is recorded as L* (81.24), a* (7), b* (12.03). Based on the colour space and the L*a*b* value, the powder sample was determined to have a desert sand colour (light orange yellow). The CIELAB colour space of lemon is recorded as L* (69.13), a* (8.3), b* (14.78). Based on the colour space and L*a*b* value, the powder sample is determined to have Khaki colour (gray yellow). The CIELAB colour space of tangerines is recorded as L* (79.18), a* (6.98), b* (13.42). Based on the colour space and the L*a*b* value, the powder sample was determined to have a desert sand colour (light orange yellow). The CIELAB colour space of oranges is recorded as L* (69.56), a* (9), b* (12.47). Based on the colour space and L*a*b* value, the powder sample is determined to have Khaki colour (grey yellow). The CIELAB colour space of kumquats is recorded as L* (72.75), a* (8.43), b* (17.88). Based on the colour space and L*a*b* value, the powder sample is determined to have a tan colour (light brown). Image of pectin powder sample and colour space values of pomelos (A), lemon (B), tangerine (C), orange (D) and kumquat (E) is shown in Fig. 3.

DE and MI values of pectin: The characterization of the pectin components was evaluated by quantifying the pectin composition, calculating the DE and MI coefficients and show in Table-4. Methoxyl index (MI) represents the methylation ratio of pectin, which is the mass percent of the methoxyl group (-O-CH_3) to the total molecular weight. Pectin quantification results of pomelos peel showed that 1 g of pectin powder have 333.84 mg of pure pectin. Pectin powder has a DE and MI coefficient of 28.45% and 2.11%, respectively. The pectin from pomelo has a DE index of 31.5% and MI of 6.4%; belonging to the LM pectin class [33]. The results showed that pectin powder from pomelo peel of pectin LM (low-methoxyl) contains fewer esters and as a result freer acid group (-COOH). These groups are negatively charged in neutral or acidic pH (-COO–). Pectin quantification results of lemon peel showed that 1 g of pectin powder have 132.66 mg of pure pectin. Pectin powder has a DE and MI coefficient of 53.82% and 1.16%, respectively. According to Karim *et al*. [34], DE is 38.5% on the lemon peel, which indicates that the pectin from the lemon peel belongs to the low methoxyl (LM) category [34]. The results show that the pectin powder from lemon peel belongs to the

TABLE-3
MOISTURE VALUE, PECTIN EXTRACTION EFFICIENCY, COLOUR VALUE ACCORDING TO CIELAB COLOUR SPACE OF ORANGE, LEMON, TANGERINE, GRAPEFRUIT, KUMQUATS PEEL EXTRACTS

Parameter	Pomelos	Lemon	Tangerine	Orange	Kumquat
Yield pectin (%)	37.08	46.07	38.55	39.17	41.78
Moisture (%)	3.00	1.30	8.20	1.78	1.61
Colour, CieLab coordinates					
L*	81.24	69.13	79.18	69.56	72.75
a*	7.00	8.30	6.98	9.00	8.43
b*	12.03	14.78	13.42	12.47	17.88

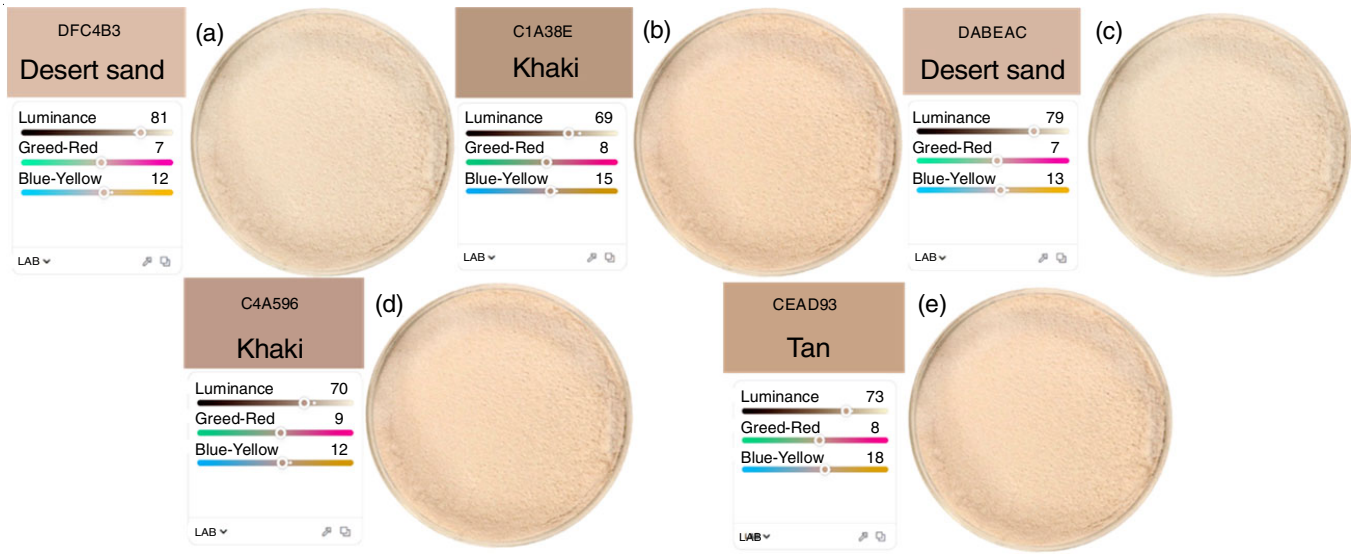

Fig. 3. Image of pectin powder sample and colour space values of pomelos (a), lemon (b), tangerine (c), orange (d), kumquat (e)

TABLE-4
DEGREE OF ESTERIFICATION VALUES, METHOXYL INDEX VALUES, PURE PECTIN CONTENT AND TYPE OF PECTIN OF ORANGE, LEMON, TANGERINE, POMELO, KUMQUAT PEELS

Parameter	Pomelo	Lemon	Tangerine	Orange	Kumquat
Degree of esterification (%)	28.45	53.82	43.81	45.23	54.29
Methoxyl index values (%)	2.11	1.16	1.01	1.43	1.24
Pure pectin (mg g^{-1})	333.84	132.66	119.97	195.41	148.67
Type of pectin	LM	HM	LM	LM	HM

high-methoxyl (HM) pectin containing more esters due to the DE coefficient > 50%. This is a new point in the study of the pectin composition of lemons. Pectin quantification results of orange peel was show that 1 g of pectin powder have 195.41 mg of pure pectin. Pectin powder has a DE and MI coefficient of 36.29% and 1.43%, respectively. According to Duwee *et al.* [35], the DE on orange peels is 59%, which indicates that the pectin from the orange peel belongs to the HM category. The results showed that pectin powder from orange peel belonging to pectin LM contains fewer esters due to the coefficient of DE < 50% and MI < 7%. Pectin quantification results of tangerine peel showed that 1 g of pectin powder have 119.97 mg of pure pectin. In the study of Twinomuhwezi *et al.* [36], pectin powder from tangerine has an MI coefficient from 10% to 13% (MI > 7%). This shows that the pectin powder from tangerine peel belongs to the type of pectin HM containing more esters. But the results for tangerine peel pectin of LM pectin contain fewer esters due to DE < 50% and MI < 7% and result in more free acid groups (-COOH). In case of kumquat peel, 1 g of pectin powder have 148.67 mg of pure pectin and indicate that kumquat peel pectin is LM (low-methoxyl) pectin as it contain fewer esters due to DE < 50% and MI < 7% and result in more free acid groups (-COOH). The results showed that pectin powder from kumquat peel has negatively charged groups in neutral pH or acidic pH (-COO-). For lemon peer, the pectin powder is classified as HM and other samples are LM. But the content of pure pectin in pomelos peel is found to be the highest. So for practical applications, pectin from pomelos peel is a good choice.

Characteristic studies of pectin: Structural characteristics of pectin from orange, lemon, tangerine, pomelo and kumquat were analyzed through scanning electron microscope (SEM), X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopies. The morphology of pectin samples of oranges, lemons, tangerines, grapefruits and kumquats is shown in Fig. 4. Clear images of the pectin samples were displayed at a size of 100 µm, highlighting the clear differences between them. Moreover, the shape of pectin is polygonal with different sizes. It can be seen that the particle size of the clog sample is larger than the other samples. Among them, grapefruit and tangerine samples show almost small sizes.

The crystal structure of pectin from orange, lemon, tangerine, pomelo and kumquat peels was evaluated by XRD technique. The results (Fig. 5) showed that the pectin sample has an amorphous structure with characteristic peaks at 2θ = 21° and 16°, which almost similar to the reported values [37] confirming pure pectin. The two pectin samples from kumquat and tangerine peels showed the new characteristic peaks at 2θ = 15°, 24°, 30°, 36°, 38° and 44°. It was observed that when the DE coefficient is > 50%, the pectin molecules form an ordered arrangement or lattice crystallization [38,39]. This partly shows that the DE coefficient affects the crystallinity of pectin.

The functional groups present in the pectin obtained from orange, lemon, tangerine, pomelo, kumquat peels are shown in Fig. 6 using the FTIR method. The FTIR results show a broad band in the range 3600-3300 cm^{-1} which is to due to the stretching vibration of the -OH group attributed to intermolecular and intramolecular hydrogen or moisture present in the powder

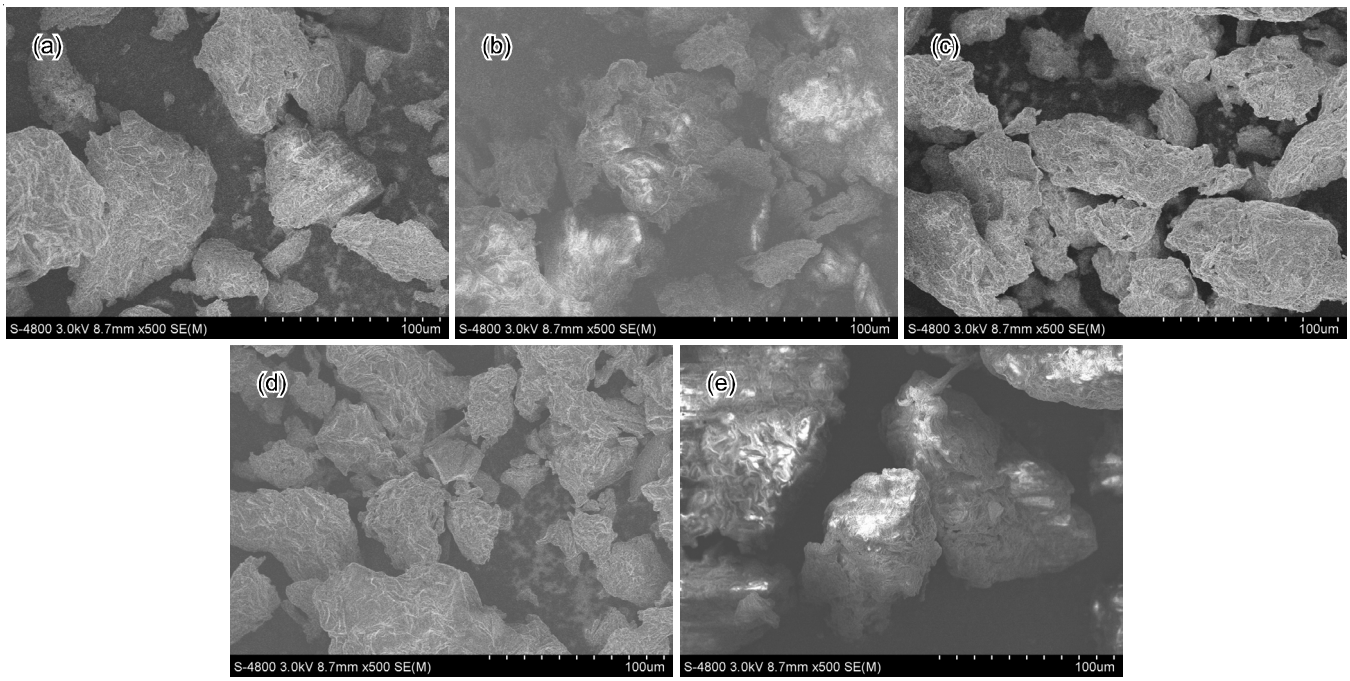

Fig. 4. SEM photographs of pectinfrom orange (a), lemon (b), tangerine (c), pomelo (d), kumquat (e) peels

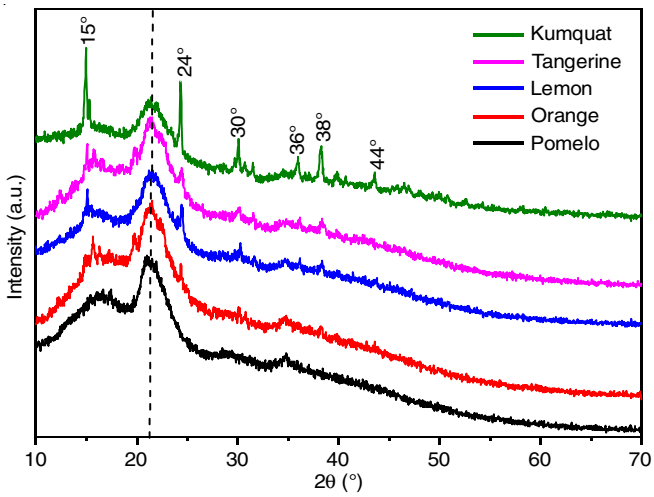

Fig. 5. XRD pattern of pectin powder from orange, lemon, tangerine, pomelo, kumquat peels

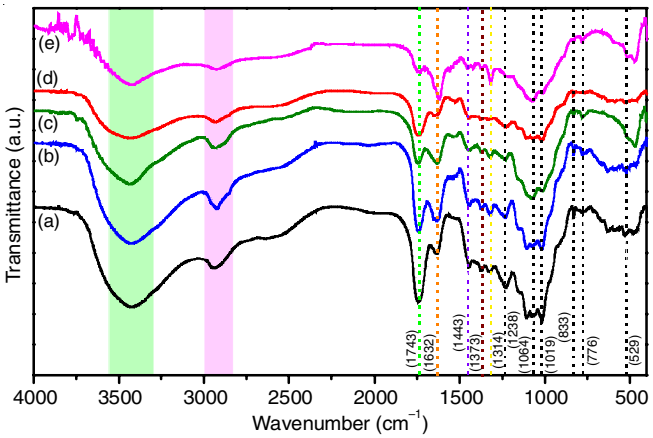

Fig. 6. FTIR spectra of pectin powder from pomelo (a), lemon (b), kumquat (c), orange (d), tangerine (e) peels

sample [40]. The peaks at 2924, 1443, 1373 and 1314 cm^{-1} are due the stretching and bending vibrations of the saturated CH group, including CH, CH_2 and CH_3. While the peaks at 1743 and 1632 cm^{-1} are due to the presence of C=O belonging to the free carboxyl group and the esterified group [41]. The adsorption band between 1250 cm^{-1} and 1000 cm^{-1} is mainly related to the glycosidic bonds (C–O–C functional groups) in galacturonic acid molecules. The peaks below 1000 cm^{-1} are attributed to the bending vibrations of C–C–H, C–O–H groups and out-of-plane vibrations of hydroxyl. In addition, the peak at 1200~500 cm^{-1} is considered the only defined carbohydrate region for all types of pectin [41,42]. Wandee *et al.* [43] also reported that when the DE coefficient is higher, the intensity of the OH group decreases and the intensity of carboxyl group increases, which is attributed to an increase in the methoxy content of pectin.

Conclusion

Extracts from oranges, lemons, tangerines, pomelos and kumquats were evaluated for their moisture colour, biological activity and antibacterial capacity. Raw material powder after preliminary processing tends to turn golden brown with moisture content below 10%. The antioxidant capacity of the extract from low to high (orange > mandarin > pomelos > lemon > kumquat) was clearly investigated. In addition, the total polyphenol and flavonoid content of pomelos and mandarin was found to be higher than that of other extracts. The antibacterial ability was also evaluated based on four intestinal-specific strains including *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It was found that orange peel extract from SE method is the most resistant to the studied bacterial strains. Besides, the extracts all have antibacterial ability but the ability was moderate. For citrus peel extracts, the polyhenol and flavonoid contents isolated from the two methods (SE and

UE) were almost the same. The pectin composition in the peels of oranges, lemons, tangerines, grapefruits and kumquats have also been specifically evaluated. All types of pectin powders have a DE coefficient < 50% corresponding to pectin LM, especially pectin from lemon peel gives a DE coefficient > 50% corresponding to pectin HM. The content of pure pectin present in pomelo samples is considered to be the highest.

ACKNOWLEDGEMENTS

This research was fund by a grant from the Science and Technology Development Foundation of Nguyen Tat Thanh University (No: 2023.01.84/HĐ-KHCN).

CONFLICT OF INTEREST

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

REFERENCES

1. M.S. Appelhans, M.J. Bayly, M.M. Heslewood, M. Groppo, G.A. Verboom, P.I. Forster, J.A. Kallunki and M.F. Duretto, *Taxon*, **70**, 1035 (2021); https://doi.org/10.1002/tax.12543
2. C.M. Morton and C. Telmer, *Ann. Mo. Bot. Gard.*, **99**, 620 (2014); https://doi.org/10.3417/2010034
3. R.L. Rouseff, P. Ruiz Perez-Cacho and F. Jabalpurwala, *J. Agric. Food Chem.*, **57**, 8115 (2009); https://doi.org/10.1021/jf900112y
4. D. Panwar, P.S. Panesar and H.K. Chopra, *Food Rev. Int.*, **37**, 91 (2021); https://doi.org/10.1080/87559129.2019.1695834
5. B. Satari and K. Karimi, *Resour. Conserv. Recycling*, **129**, 153 (2018); https://doi.org/10.1016/j.resconrec.2017.10.032
6. N. Mahato, K. Sharma, M. Sinha and M.H. Cho, *J. Funct. Foods*, **40**, 307 (2018); https://doi.org/10.1016/j.jff.2017.11.015
7. M. Boukroufa, C. Boutekedjiret, L. Petigny, N. Rakotomanomana and F. Chemat, *Ultrason. Sonochem.*, **24**, 72 (2015); https://doi.org/10.1016/j.ultsonch.2014.11.015
8. H. Alasalvar, M. Kaya, S. Berktas, B. Basyigit and M. Cam, *Int. J. Food Sci. Technol.*, **58**, 2060 (2023); https://doi.org/10.1111/ijfs.15925
9. S. Šafranko, I. Corkovic, I. Jerkovic, M. Jakovljevic, K. Aladic, D. Šubaric and S. Jokic, *Foods*, **10**, 1043 (2021); https://doi.org/10.3390/foods10051043
10. H.K. Sandhu, P. Sinha, N. Emanuel, N. Kumar, R. Sami, E. Khojah and A.A.M. Al-Mushhin, *Separations*, **8**, 244 (2021); https://doi.org/10.3390/separations8120244
11. B. Singh, J.P. Singh, A. Kaur and N. Singh, *Food Res. Int.*, **132**, 109114 (2020); https://doi.org/10.1016/j.foodres.2020.109114
12. N. M'hiri, I. Ioannou, M. Ghoul and N.M. Boudhrioua, *Food Rev. Int.*, **30**, 265 (2014); https://doi.org/10.1080/87559129.2014.924139
13. A. Jagannath and R. Biradar, *J. Food Chem. Nanotechnol.*, **5**, 56 (2019); https://doi.org/10.17756/jfcn.2019-072
14. S. Rafiq, R. Kaul, S.A. Sofi, N. Bashir, F. Nazir and G. Ahmad Nayik, *J. Saudi Soc. Agric. Sci.*, **17**, 351 (2018); https://doi.org/10.1016/j.jssas.2016.07.006
15. C.Y. He, Y.M. Sun, G.H. Wu and R. Chen, *Guangpuxue Yu Guangpu Fenxi*, **21**, 719 (2001).
16. N. Koolaji, B. Shammugasamy, A. Schindeler, Q. Dong, F. Dehghani and P. Valtchev, *Curr. Dev. Nutr.*, **4**, nzaa025 (2020); https://doi.org/10.1093/cdn/nzaa025
17. W. Hu, S. Chen, D. Wu, K. Zhu and X. Ye, *Food Hydrocoll.*, **121**, 106952 (2021); https://doi.org/10.1016/j.foodhyd.2021.106952
18. M.C. Nancy Picot-Allain, A. Amiri-Rigi, K. Abdoun-Ouallouche, L. Aberkane, A. Djefal-Kerrar, M.F. Mahomoodally and M.N. Emmambux, *Food Biosci.*, **46**, 101550 (2022); https://doi.org/10.1016/j.fbio.2022.101550
19. T. Qi, J. Ren, X. Li, Q. An, N. Zhang, X. Jia, S. Pan, G. Fan, Z. Zhang and K. Wu, *Carbohydr. Polym.*, **309**, 120682 (2023); https://doi.org/10.1016/j.carbpol.2023.120682
20. N.P.M. Tô and N.V. Muoi, *The Univ. J. Sci.*, **57**, 21 (2021).
21. A. Montero-Calderon, C. Cortes, A. Zulueta, A. Frigola and M.J. Esteve, *Sci. Rep.*, **9**, 16120 (2019); https://doi.org/10.1038/s41598-019-52717-1
22. S.Q. Liew, W.H. Teoh, R. Yusoff and G.C. Ngoh, *Chem. Eng. Process.*, **143**, 107586 (2019); https://doi.org/10.1016/j.cep.2019.107586
23. E. Gómez-Mejía, N. Rosales-Conrado, M.E. León-González and Y. Madrid, *Food Chem.*, **295**, 289 (2019); https://doi.org/10.1016/j.foodchem.2019.05.136
24. W. He, X. Li, Y. Peng, X. He and S. Pan, *Molecules*, **24**, 242 (2019); https://doi.org/10.3390/molecules24020242
25. P.V. Hung, N.H.Y. Nhi, L.Y. Ting and N.T. Lan Phi, *J. Chem.*, **2020**, 1043251 (2020); https://doi.org/10.1155/2020/1043251
26. Y. Khane, K. Benouis, S. Albukhaty, G.M. Sulaiman, M.M. Abomughaid, A. Al Ali, D. Aouf, F. Fenniche, S. Khane, W. Chaibi, A. Henni, H.D. Bouras and N. Dizge, *Nanomaterials*, **12**, 2013 (2022); https://doi.org/10.3390/nano12122013
27. S. Dhushane and T. Mahendran, *J. Univ. Ruhuna*, **8**, 49 (2020); https://doi.org/10.4038/jur.v8i1.7964
28. A. Ulmillah, A. Alghifari and N. Widiani, *Biol. Mad. Nat. Prod. Chem.*, **12**, 323 (2023); https://doi.org/10.14421/biomedich.2023.121.323-328
29. L.-Y. Lin, C.-Y. Huang, K.-C. Chen and R.Y. Peng, *Hortic. Environ. Biotechnol.*, **62**, 377 (2021); https://doi.org/10.1007/s13580-020-00325-8
30. M. Anticona, J. Blesa, A. Frigola and M.J. Esteve, *Foods*, **9**, 811 (2020); https://doi.org/10.3390/foods9060811
31. N. Phuyal, P.K. Jha, P.P. Raturi and S. Rajbhandary, *Scient. World J.*, **2020**, 8780704 (2020); https://doi.org/10.1155/2020/8780704
32. S. Suri, A. Singh, P.K. Nema, S. Malakar and V.K. Arora, *Food Biosci.*, **48**, 101789 (2022); https://doi.org/10.1016/j.fbio.2022.101789
33. H.K.N. Nguyen, T.T. Tran and L.G. Bach, *The Univ. J. Sci.*, **59**, 66 (2023).
34. R. Karim, K. Nahar, F.T. Zohora, M.M. Islam, R.H. Bhuiyan, M.S. Jahan and M.A.A. Shaikh, *Carbohydr. Polym. Technol. Appl.*, **4**, 100258 (2022); https://doi.org/10.1016/j.carpta.2022.100258
35. Y.S. Duwee, P.L. Kiew and W.M. Yeoh, *J. Food Meas. Charact.*, **16**, 1710 (2022); https://doi.org/10.1007/s11694-022-01305-5
36. H. Twinomuhwezi, A.C. Godswill and D. Kahunde, *Am. J. Phys. Sci.*, **1**, 17 (2020); https://doi.org/10.47604/ajps.1049
37. R. Supreetha, S. Bindya, P. Deepika, H.M. Vinusha and B.P. Hema, *Results Chem.*, **3**, 100156 (2021); https://doi.org/10.1016/j.rechem.2021.100156
38. F.M. Carbinatto, A.D. de Castro, B.S.F. Cury, A. Magalhães and R.C. Evangelista, *Int. J. Pharm.*, **423**, 281 (2012); https://doi.org/10.1016/j.ijpharm.2011.11.042
39. A. Bagliotti Meneguin, B. Stringhetti Ferreira Cury and R.C. Evangelista, *Carbohydr. Polym.*, **99**, 140 (2014); https://doi.org/10.1016/j.carbpol.2013.07.077
40. R.K. Mishra, M. Datt and A.K. Banthia, *AAPS PharmSciTech*, **9**, 395 (2008); https://doi.org/10.1208/s12249-008-9048-6
41. Z. Sun, S. Wang, C. Zhou, Z. Ma and F. Qian, *Biomass Convers. Biorefin.*, (2023); https://doi.org/10.1007/s13399-023-04200-1
42. D. Demir, S. Ceylan, D. Göktürk and N. Bölgen, *Polym. Bull.*, **78**, 2211 (2021); https://doi.org/10.1007/s00289-020-03208-1
43. Y. Wandee, D. Uttapap and P. Mischnick, *Food Hydrocoll.*, **87**, 237 (2019); https://doi.org/10.1016/j.foodhyd.2018.08.017