Special topic paper

Stella Chintua Ortutu*, Matthew Olaleke Aremu, Grace Otobo and Ugomma Chibuzo Onyeije

Health benefit of lipid composition of orange (*Citrus sinensis*) fruit pulp at different maturation stages

https://doi.org/10.1515/pac-2023-1101

Abstract: The Sustainable Development Goal (SDG) 3, which was adopted by all United Nations member states in 2015, is 'Good Health and Wellbeing'. To contribute in the actualization of this goal, the lipid composition of unripe (UR), about to ripe (AR), and ripe (RP) *Citrus sinensis* fruit pulps were evaluated using standard analytical techniques. The result showed that the same fatty acids and phospholipids were found in all the fruit pulps examined. The most abundant fatty acid in the fruit pulps was palmitoleic acid with concentration (%) of 26.48, 27.82 and 27.14 for UR, AR, and RP samples respectively. This was followed by oleic acid (25.36 %, 29.13 %, 28.66 %), palmitic acid (25.98 %, 20.14 %, 21.66 %), linoleic acid (12.30 %, 11.18 %, 11.33 %), linolenic acid (7.52 %, 8.71 %, 8.19 %), and stearic acid (1.95 %, 2.73 %, 2.72 %) for UR, AR, and RP fruit pulps respectively. The samples contain healthy saturated and unsaturated fatty acid with the concentration of unsaturated fatty acid (71.75 %, 76.92 %, 75.40 %) being prominent in UR, AR, and RP samples respectively. The most prominent phospholipids (mg/100 g) in the fruit pulps was phosphatidylethanolamine with concentrations of 5.86, 6.47, and 6.03 for AR, UR, and RP samples respectively. This was followed by phosphatidylcholine (4.02 mg/100 g, 4.52 mg/100 g, 4.22 mg/100 g), phosphatic acid (3.59 mg/100 g, 4.02 mg/100 g, 3.89 mg/100 g), diphosphatidylglycerol (3.38 mg/100 g, 3.79 mg/100 g, 3.59 mg/100 g), phosphatidylinositol (1.92 mg/100 g, 2.24 mg/100 g, 2.21 mg/100 g), phosphatidylserine (1.86 mg/100 g, 2.08 mg/100 g, 1.91 mg/100 g) and phosphatidylglycerol (1.07 mg/100 g, 1.21 mg/100 g, 1.18 mg/100 g) for UR, AR and RP fruit pulps respectively. The result revealed that *Citrus sinensis* is a healthy low fat food at every maturation stage and that the fatty acid and phospholipid composition increased as the fruit pulp ripened.

Keywords: Citrus sinensis; fatty acids; fruit pulps; maturation stages; phospholipids; VCCA-2023.

Introduction

There is a recent interest in the lipid composition of fruits and vegetables due to consumer concern with the saturated and unsaturated fatty acid ratio of their diet. The interest of consumers is mostly on essential fatty acids, with emphasis on the health potential of polyunsaturated fatty acids. It is considered that these fatty acids play a natural preventive role in cardiovascular disease and in the alleviation of some health problems, basically because they promote the reduction of both total and high density lipoprotein (HDL) cholesterol [1]. Fruits in

Article note: A collection of invited papers based on presentations at the Virtual Conference on Chemistry and its Applications 2023 (VCCA-2023).

***Corresponding author: Stella Chintua Ortutu**, Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, Nnamdi Azikiwe University, Awka, Nigeria, e-mail: sc.ortutu@unizik.edu.ng

Matthew Olaleke Aremu, Department of Chemistry, Faculty of Chemical Sciences, Federal University Lafia, PMB 146, Lafia, Nasarawa State, Nigeria

Grace Otobo and Ugomma Chibuzo Onyeije, Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, Nnamdi Azikiwe University, Awka, Nigeria

general are not rich in lipids with the exception of avocado and olives that store large amount of triacylglycerols [2]. The major fatty acids in fruits include palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids. The lipid content of fruits influences the stability of the fruit juice and markedly modifies the taste of the juice after pasteurization and storage [2]. Phospholipids are essential components of every living cell. Due to their wide structural diversity, they can be used as quantitative biomarkers [3, 4]. They are also used to characterize microbial community. This is because they are not found in storage products or in dead cells because their phosphate group is quickly hydrolyzed to diglycerides after the death of the cell [4, 5]. The major phospholipids that constitute the biomolecules include phosphatidylcholine, phosphatidylethanoalamine, phosphatidylglycerol and phosphatidylinositol [2]. In addition, metabolic intermediates of phospholipids such as phosphatidic acid, diacylglycerols and free fatty acids are also present in the membrane in lower amounts [6].

Orange (*Citrus sinensis*) is a commonly consumed fruit in Nigeria. It belongs to the family Rutaceae and originated from Southern China, Northeast India and Myanmar This fruit is highly consumed worldwide either as fresh produce or in the form of juice and the peel is usually discarded as primary waste in the environment [7]. The fruit pulp represents about 40 %–50 % of wet fruit mass and is found to be a potential source of bioactive components such as ascorbic acid, carotenoids, phenolic compounds and flavonoids [7, 8]. Citrus fruit maturation depends on the internal changes occurring in the fruit flesh as well as the external fruit peel coloration which occur during fruit development, growth, and maturity [9]. Commercial maturity indices in citrus fruit are highly variable depending on the ripening stage, environmental conditions, growing region, and varieties [10]. Ripe *Citrus sinensis* is extensively processed by the manufacturing industry in order to obtain natural juices, pulps, and candies, and these extracts have been demonstrated to be a vital dietary source of vitamin C, liminoids, synephrine, hesperidin, polyphenols, pectins, calcium, potassium, thiamine, niacin, and magnesium [11].

To our knowledge, there is no available information on the lipid content of the pulp extracts of unripe, about to ripe, and ripe *Citrus sinensis*. To also contribute to the actualization of 2030 Sustainable Developmental Goals (SDGs) adopted by all United Nations Member States Number 3, the present study evaluated the fatty acid and phospholipid composition of *Citrus sinensis* fruit pulps at different maturation stages.

Materials and methods

Collection of samples

Orange (*Citrus sinensis*) fruits were harvested from its tree from Enugu State of Nigeria. Based on visual observation of color, texture and flavor, the fruits were categorized into unripe (UR), about to ripe (AR), and ripe (RP). The fruits were sliced into halves with a sharp knife and their seeds removed. The categorized and sliced orange fruits were taken to the Medicinal Department of National Institute of Pharmaceutical Research and Development (NIPRED) Idu, FCT, Abuja for further treatment and analysis.

Preparation and sample treatment

The categorized and sliced fruits were weighed, homogenized using a domestic blender, filtered using a cheese cloth and freeze-dried using AMSCO-AQUA LYOVAC GT2/GT2-E freeze drier. The freeze dried samples were stored in an air-tight sample bottles and used for the analysis.

Extraction of oil

Crude fat was extracted based on the method described by [12]. The extraction flask of 250 mL capacity was dried in the oven at 105 °C, transferred to the desiccator to cool to the laboratory temperature and the weight of the flask was measured. About 2.0 g of each categorized sample was weighed into the labeled porous thimble. 200 mL of

petroleum ether was measured and added to the dried 250 mL extraction flask. The covered porous thimble with the sample was placed in the condenser of the Soxhlet extractor arrangement that has been assembled. The sample was extracted for 5 h the porous thimble was removed with care and the petroleum ether in the top container (tube) was collected for recycling and reuse. The extraction flask was removed from the heating mantle arrangement when it was almost free of petroleum ether. The extraction flask with the oil was oven-dried at 105 ° C for a period of 1 h the flask containing the dried oil was cooled in the desiccator and the weight of the cooled flask with the dried oil was measured.

Fatty acid analysis

The crude fat was converted to methyl ester using the method described by [12, 13]. The extracted fat content (50 mg) of each categorized sample was saponified (esterified) for 5 min at 95 °C with 3.4 mL of 0.5 M KOH in dry methanol. The mixture was neutralized by using 0.7 M HCl, 3 mL of 14 % boron trifluoride in methanol was added. The mixture was heated for 5 min at the temperature of 90 °C to achieve complete methylatio. The fatty acid methylesters were extracted from the mixture with redistilled n-hexane in triplicate. The content was concentrated to 1.0 mL for GC analysis and 1.0 µL was injected into the injection port of GC. The injection port and the detector were maintained at 310 °C and 350 °C, respectively while the initial column temperature was 250 °C rising at 5 °C/min to a final temperature of 310 °C. A polar (HPINNO Wax) capillary column (30 m/0.25 mm/0.25 µm) was used to separate the esters. The peaks were identified by comparison with standard fatty acid methyl esters obtained from Sigma Chemical Co (St Louis MO. USA). Three (3) determinations were made for each of the categorized sample.

Phospholipids analysis

The modified method of Aremu et al. [14] was employed in the phospholipids analysis of the categorized crude fat. The extracted crude fat (0.01 g) was added to the test tubes. To ensure complete drying of the oil for phospholipid analysis, the solvent was completely removed by passing the stream of nitrogen gas on the oil. 0.4 mL of chloroform was added to the content of the tube and it was followed by the addition of 0.10 mL of the chromogenic solution. The content of the tube was heated at the temperature of 100 °C in a water bath for about 1 min 20 s. The content was allowed to cool to the laboratory temperature and 5 mL of the hexane was added and the tube with its content shaken gently several times. The solvent and the aqueous layers were allowed to be separated. The hexane layer was recovered and allowed to be concentrated to 1.0 mL for GC analysis using the pulse flame photometric detector.

Statistical analysis

The statistical analysis used in the expression of data were mean determination, standard deviation and coefficient of variation in percentage.

Results and discussion

Results

See Tables 1–3.

Table 1: Fatty acid composition (%) of *Citrus sinensis* at different maturation stages.

Fatty acid (%)	UR	AR	RP	Mean	SD	CV (%)
Myristic acid (8:0)	0.09	0.00	0.03	0.04	0.05	125.00
Palmitic acid (16:0)	25.98	20.14	21.66	22.59	3.03	13.41
Palmitoleic acid (16:1)	26.48	27.82	27.14	27.15	0.67	2.47
Margaric acid (17:0)	0.10	0.09	0.08	0.09	0.01	11.11
Stearic acid (18:0)	1.95	2.73	2.73	2.47	0.45	18.22
Oleic acid (18:1)	25.36	29.13	28.66	27.72	2.05	7.40
Linoleic acid (18:2)	12.30	11.18	11.33	11.60	0.61	5.26
Linolenic acid (18:3)	7.52	8.71	8.19	8.14	0.60	7.37
Arachidonic acid (20:4)	0.06	0.05	0.05	0.05	0.01	20.00
Behenic acid (22:0)	0.06	0.05	0.05	0.05	0.01	20.00
Erucic acid (22:1)	0.03	0.03	0.03	0.03	0.00	0.00
Lignoceric acid (24:0)	0.07	0.06	0.06	0.06	0.01	16.67

SD, standard deviation; CV, coefficient of variation; UR, unripe; AR, about to ripe; RP, ripe.

Table 2: Quality parameter of *Citrus sinensis* fruit pulp at different maturation stages.

Fatty acid (%)	UR	AR	RP	Mean	SD	CV (%)
TSFA	28.25	23.07	24.60	25.31	2.66	10.51
TUFA	71.75	76.92	75.40	74.69	2.66	3.56
TMUFA	51.87	56.98	55.83	54.89	2.68	4.88
TPUFA	19.88	19.94	19.57	19.80	0.20	1.01
TEFA	19.88	19.94	19.57	19.80	0.20	1.01
∑n-3PUFA	7.58	8.76	8.24	8.19	0.59	7.20
∑n-6PUFA	12.30	11.18	11.33	11.60	0.61	5.26
∑n-6PUFA/∑n-3PUFA	1.62	1.28	1.38	1.43	0.18	12.59
O/L ratio	2.06	3.34	2.53	2.64	0.65	24.62

SD, standard deviation; CV, coefficient of variation; UR, unripe; AR, about to ripe; RP, ripe; TSFA, total saturated fatty acid; TUFA, total unsaturated fatty acid; TMUFA, total monounsaturated fatty acid; TPUFA, total polyunsaturated fatty acid; TEFA, total essential fatty acid; ∑n-3PUFA, total omega-3 PUFA; ∑n-6PUFA, total omega-6 PUFA; ∑n-6PUFA/∑n-3PUFA, ratio of omega-6 to omega-3; O/L, ratio of oleic acid to linoleic acid.

Table 3: Phospholipids level (mg/100 g) of *Citrus sinensis* at different maturation stages.

Phospholipids (mg/100 g)	UR	AR	RP	Mean	SD	CV (%)
Phosphatidylethanolamine (PE)	5.86	6.47	6.03	6.12	0.32	5.23
Phosphatidylcholine (PC)	4.02	4.52	4.22	4.25	0.25	5.88
Phosphatidylglycerol (PG)	1.07	1.21	1.18	1.15	0.07	6.09
Phosphatidylserine (PS)	1.86	2.08	1.91	1.95	0.12	6.15
Phosphatidylnositol (PN)	1.92	2.24	2.21	2.12	0.18	8.49
Diphosphatidylglycerol (DPG)	3.38	3.79	3.59	3.59	0.21	5.85
Phosphatidic acid (PA)	3.59	4.02	3.89	3.83	0.22	5.74
Total	21.70	24.33	23.03	23.01	1.37	43.43

SD, standard deviation; CV, coefficient of variation; UR, unripe; AR, about to ripe; RP, ripe.

Discussion

The results of the fatty acid composition of orange (*Citrus sinensis*) shows that UR, AR and RP fruit pulps possess high oleic acid (18:1) concentration (%) of 25.36, 29.13 and 28.66 respectively (Table 1). This was followed by palmitoleic acid (16:1) with values of 26.48, 27.82 and 27.14 for UR, AR and RP samples respectively. The most

prominent saturated fatty acid in the fruit pulps was found to be palmitic acid (16:0) with content of 25.98 % for UR fruit pulp, 20.14 % for AR fruit pulp and 21.66 % for RP fruit pulp. This was followed by stearic acid (18:0) with concentrations of 1.95 % for UR, 2.73 % for AR and 2.72 % for RP. The palmitic acid content (%) of the fruit pulps (UR 25.98; AR 20.14; RP 21.66) was found to be comparable to that found in guava (UR 26.36; AR 23.71; RP 23.76) and greater than that found in *Persea americana* pulp (18.76 %) and seed (11.74 %), *Artocarpus altilis* (11.41 %) and raw tiger nut (12.96) as reported by [12, 14–16]. The linoleic acid values of the fruit pulps (UR 12.30 %; AR 11.18 %; RP 11.33 %) was found to be low when compared to that found in *Sorghum bicolor* (50.31 %), *Persea americana* pulp (23.02 %) and seed (38.15 %) as reported by [12, 17]. The fatty acid result of *Citrus sinensis* at different maturation stages is in agreement with that of Chivandi [6] who reported that the major fatty acids in fruits are palmitic, stearic, palmitoleic, oleic, linoleic and linolenic.

Table 2 present the distribution of the quality fatty acids in *Citrus senensis* fruit pulp at different maturation stages. It was observed that the percentage of the total saturated fatty acid (TSFA) found in the unripe (UR) fruit pulp was higher than that found in the about to ripe (AR) and the ripe (RP) fruit pulps while the percentage of the total unsaturated fatty acid (TUFA), and the total essential fatty acid (TEFA) was found to be highest in the AR fruit pulp and lowest in the UR fruit pulp. The total saturated fatty acid (TSFA) content (%) was found to be 28.25, 23.07 and 24.60 for UR, AR and RP fruit pulps respectively. These values are higher than the TSFA value of 20.17 % and 20.50 % found in germinated Sorghum [17] and boiled tigernut [16] respectively and lower than 38.74 %, 98.61 % and 40.00 % found in *Pearsea americana* pulp [12], mango kernel [18], and tonoplast pineapple [19]. Total unsaturated fatty acid (TUFA) of 71.75 %, 76.92 % and 75.40 % for UR, AR and RP *Citrus sinensis* fruit pulps was found to be very high when compared to that found in fermented African locust bean (56.9 %) [20], processed tilapia fish (59.3 %) and mesquite bean (56.90 %) [21]. The most abundant PUFA was linoleleic acid in all the fruit pulps examined.

The phospholipid concentration (mg/100 g) of *Citrus sinensis* fruit pulps at different maturation stages are shown in Table 3. The same type of phospholipids was present in UR, AR and RP fruit pulps but with different concentrations. The oil from AR pulp contained the highest concentration of phospholipids (24.33) followed by RP pulp (23.03) and UR pulp (21.70). The most abundant phospholipids in the fruit pulps was phosphatidylethanoalamine (PE) with values of 5.86, 6.47 and 6.03 for UR, AR and RP samples respectively. Phosphatidylcholine (PC) came second with concentrations of 4.02 for UR, 4.52 for AR and 4.22 for RP. This was followed by PA, DPG, PN, PS, and PG with concentrations ranging from 1.07 in AR (PG) sample to 4.02 in AR (PA). The result of this study is in agreement with that of Aremu et al. [12] who reported that PC and PE were the most prominent phospholipids in *Persea americana*. Phosphatidylethanoalamine (PE) is usually the most abundant phospholipids in animals and plants often amounting to about 50 % of the total and as such are building blocks of membrane bilayer [22].

The values of total monounsaturated fatty acid (TMUFA) was found to be 51.87 %, 56.98 % and 55.83 % for UR, AR and RP samples respectively. These values shows that TMUFA was the most predominant of the total unsaturated fatty acids in the fruit pulps irrespective of their maturation stages. These values are higher than that found in *Peasea americana* pulp (40.63 %) [12] and *S. bicolor* (29.24 %) [17]. TMUFA value in the about to ripe (AR) pulp was higher than that found in unripe (UR) and ripe (RP) samples. Monounsaturated fatty acid plays an important role in lowering LDL (bad) cholesterol level. Thereby, reducing the risk of heart disease and stroke. It also helps in developing and maintaining the cells. *Citrus sinensis* fruit pulp at different maturation stages will participate well in these roles. Linoleic acid (18:2) value of 12.30 % found in UR sample is higher than that found in AR (11.18 %) and RP (11.33 %) samples. Linoleic acid helps to improve the brain, heart, skin, bone and reproductive health [23]. The value of alpha-linoleic acid (18:3) in AR (8.71 %) is higher than that found in RP (8.19 %) and UR (7.52 %) samples. Alpha-linoleic acid (ALA) and linoleic acid (LA) are omega-3 and omega-6 essential fatty acids. They play important roles in membrane structure and as starting point for making hormones that regulate blood clotting, contraction and relaxation of artery walls and inflammation. As a result of these roles, they help prevent heart disease and stroke, may help control lupus, eczema and rheumatoid arthritis, and may play protective roles in cancer and other conditions. Myristic, behenic, and lignoceric are saturated acids found in all the samples in small quantities of less than 1.00 %. The total polyunsaturated fatty acids (TPUFA) in the fruit pulps ranged from 19.57 % in RP sample to 19.94 % in AR sample. This value is low when compared to that found in *Persea americana* pulp (25.84 %) and seed (40.57 %) [12]. This suggest that the cholesterol of the oil in the fruit pulps (especially the

unripe) are very low and could be useful in reducing the incidence of heart attack (atherosclerosis) caused by high intake of cholesterol.

The result of the study is in agreement with that of Writz [12] who reported that phosphatidylethanoalamine (PE) is the most abundant phospholipids in animal and plants lipids. The PE values (mg/100 g) in UR, AR and RP fruit pulps were 5.86, 6.47 and 6.03 respectively. PE is essential in the promotion of membrane fusion and fission, protein integration into membranes, conformational changes in protein structure and a precursor of other lipids. Phosphatidylcholine are key sources for biologically potent eicosanoids. Eicosanoids are enzymatic derivation of arachidonic acid, which is released from the sn-2 position of cellular membrane phospholipids by cystosolic phospholipase A_2 ($cPLA_2$) during the inflammatory response [24]. Phosphatidic acid which is the third most abundant phospholipid in the fruit pulps plays the role of signaling pathways in cell growth, production and responses to hormones in biotic and abiotic stress [25] while phosphatidylserine reduces mental stress and increases mental accuracy in young people. In elderly people, it reduces the rate of dementia and cognitive dysfunction [25]. Considering the concentration of phospholipids in *Citrus sinensis* fruit pulps at all the maturation stages examined, they may participate well in these functions.

Conclusions

The research work on the lipid composition of *Citrus sinensis* at different maturation stages showed that the fruit pulps contained healthy saturated and unsaturated fatty acids. The unripe sample had the highest concentration of saturated fatty acids while about to ripe sample had the highest concentration of MUFA. The phospholipids composition suggests that all the fruit pulps are good sources phospholipids and that the phospholipid concentration increases as the fruit pulp ripens.

Research ethics: Not applicable.
Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: The authors state no conflict of interest.
Research funding: None declared.
Data availability: Not applicable.

References

[1] P. Melgarejo, F. Artes. *J. Sci. Food Agric.* **80**, 1452 (2020), https://doi.org/10.1002/1097-0010(200008)80:10<1452::aid-jsfa665>3.0.co;2-l.
[2] C. Nieolosin, S. Cataldi, R. C. Campisi. *J. Agric. Food Chem.* **35**(6), 1023 (1987).
[3] J. Tomberg, M. Unemo, M. Ohnishi, C. Davies, R. A. Nicholas. *Am. Soc. Microbiol.* **57**(7), 3029 (2013), https://doi.org/10.1128/aac.00093-13.
[4] L. Zelles. *ScienceDirect* **35**(2), 275 (1997), https://doi.org/10.1016/s0045-6535(97)00155-0.
[5] D. C. White, W. M. Davis, J. S. Nickels, J. D. King, R. J. Bobbie. *Oecologia* **40**, 51 (1997), https://doi.org/10.1007/bf00388810.
[6] E. Chivandi, K. Erlwanger, B. Davidson. *Int. J. Integr. Biol.* **5**(2), 102 (2009).
[7] B. Sharma, D. U. Shah, J. Beaugrand. *J. Res. Pharm. and Biotech.* **1**, 40 (2018).
[8] S. C. Ortutu, M. O. Aremu. *J. Open Pharm. Sci.* **3**, 1 (2016).
[9] K. Kashyap, S. Banu. *Turk. J. Bot.* **43**(2), 173 (2019), https://doi.org/10.3906/bot-1711-45.
[10] J. Lado, M. J. Rodrigo, L. Zacarias. in *Maturity indicators and citrus fruit quality*, Stewart Postharvest Rev., Bangladesh (2014).
[11] O. S. Nwozo, E. M. Effiong, P. M. Aja, C. G. Awuchi. *Int. J. Food Prop.* **26**(1), 359 (2023), https://doi.org/10.1080/10942912.2022.2157425.
[12] M. O. Aremu, M. A. Odey, L. Labaran, C. C. Nweze, R. B. Salau, S. C. Ortutu. *Trends Med. Res.* **10**(10), 327 (2020).
[13] E. T. Akintayo, E. Bayer. *Bioresour. Technol.* **85**, 95 (2002), https://doi.org/10.1016/s0960-8524(02)00073-1.
[14] M. O. Aremu, H. Ibrahim, C. Andew. *Open Biochem. J.* **11**, 94 (2017), https://doi.org/10.2174/1874091x01711010094.
[15] S. C. Ortutu, M. O. Aremu. *Riv. Ital. delle Sostanze Grasse, La* **94**(4), 265 (2017).
[16] M. O. Aremu, H. Ibrahim, S. O. Aremu. *Pakistan J. Nutr.* **15**(15), 427 (2016), https://doi.org/10.3923/pjn.2016.427.438.
[17] M.O. Aremu, E. I. Achuri, S. C. Ortutu, O. M. Omosebi, A. Usman, J. O. Oko, L. Labaran. *Int. J. Sci.* **11**(1), 22 (2022), https://doi.org/10.18483/ijsci.2541.

[18] B. S. Ogunsina, A. S. Bhatnager, T. N. Indira, C. Radha. *J. Life Sci.* **14**(1), 177 (2012).
[19] Y. Zhou, X. Pan, H. QU, S. J. Underhil. *US Natl. Libr. Med.* **247**(5), 429 (2014), https://doi.org/10.1007/s00232-014-9650-3.
[20] M. O. Aremu, H. Ibrahim, E. Y. Awala, A. Olonisakin, O. J. Oko. *J. Chem. Eng. Chm. Res.* **2**, 817 (2015).
[21] C. O. Agbo, M. O. Aremu, O. J. Oko, P. C. Madu, S. B. Namo. *J. Nat. Sci. Res.* **4**(10), 147 (2014).
[22] K. Wirtz. *Annu. Rev. Biochem.* **60**(13), 73 (2001), https://doi.org/10.1146/annurev.bi.60.070191.000445.
[23] L. U. Guang, W. Yu, S. Yin, Z. Zhang, C. Huang, W. He, C. Wang, H. M. Shen. *MedComm* **3**(3), e150 (2022), https://doi.org/10.1002/mco2.150.
[24] N. Kanno, Y. Ueno, Y. Moritoki. *Yangtze Med.* **1**(2), 77–93 (2017).
[25] Y. W. Wang, P. J. Jones. *Int. J. Obes.* **28**(28), 941 (2004), https://doi.org/10.1038/sj.ijo.0802641.