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A Key Component Determination on Forming Fairy Tofu from the Leaf of *Premna Puberula* (Verbenaceae)

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ABSTRACT

A key component of Fairy Tofu from leaves of *Premna puberula* Pamp. was determined by immediate constituent analysis and prescription dismantlement analysis. The results showed that pectin in leaves of *P. puberula* was the key component as forming Fairy Tofu. It was because the highest component was pectin in Fairy Tofu except water, the pectin extracting directly from the leaves of *P. puberula* could be formed the jelly like Fairy Tofu, and the leaves which pectin was hydrolyzed by pectinase could not be made Fairy Tofu.

INTRODUCTION

Premna puberula Pamp. is a perennial deciduous shrub, a plant both edible and medicinal, belong to the Verbenaceae and *Premna*. It distributed mainly in southwest, east, south central of China, and grew on the hillside or in the roadside underbrush at the altitude of 700 m to 1,800 m (Yunnan, 1977). Its leaves and roots can be used as medicine, which was very effective on clearing heat, removing toxicity, regulating menstruation and tonifying yang (Institute, 1983), and for the treatment of dropsy, carbuncle, menoxenia, impotence, rheumatic arthritis, etc. (Editorial, 1999).. Meanwhile, people often used the leaves of *P. puberular* to process and make a cool tofu, commonly known as Fairy Tofu, it contained the right amount of soluble sugars and proteins, a variety of essential amino acids, abundant vitamin C and a large number of mineral elements, and with delicious taste and emerald color (Gao et al., 2003). Fairy tofu is a traditional natural food with relieve summer heat, and has broad prospects for development and utilization because of its high nutritional value, simple processing procedure, less investment in equipments, and so on. However, relevant researches of *Premna* were mainly focused on the development and utilization of pectins (Liu et al., 2006; Ma 2007; Ning, 2010; Wang et al., 2011), and the reports about the basic components and the formation mechanism of Fairy Tofu were less. In this paper, authors explored the key component of forming Fairy Tofu, so as to lay a foundation for the industrialization of *P. puberula.*

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MATERIALS AND METHODS

Plant materials

Premna puberula Pamp. (identified by Professor Yuanxin Xiong of Guizhou University) were collected from Gelao and Miao Autonomous County of Wuchuan in Guizhou Province, China. All plants were potted in sandy-loam (sand accounted for 15%) and grew in the light transmission rate of 20% to 25% shade net shed.

Methods

Preparation of Fairy Tofu

The basic process by fresh leaves making Fairy Tofu (traditional method)

Picking the leaves of *P. puberula*, washing and airing them. Then soaking the leaves with distilled water at a ratio 1:20 (w/w) and grinding the mixtures into homogenate, filtering the homogenate by gauze and collecting the filtrate. Finally, injecting clear saturated limewater into the filtrate at the ratio of limewater and filtrate equaled 1:20 (v/v), stirring the mixtures evenly and then let it standing until to jelly into Fairy Tofu.

The basic process by dried leaves making Fairy Tofu

De-enzyming the fresh leaves of *P. puberula* for 15 to 20 min in 105℃ drying oven, and then continued drying to constant weight at 60℃. Grinding the dried leaves into powder and to let the powder passing through 60 mesh sieve. Mixing the fine powder with distilled water at a ratio of 1:50 (w/w), and adjusting pH of the mixed homogenate to 5, heating the mixtures for 60 min at 60℃ in water bath, collecting the filtrate by suction filtering. After that, injecting clear saturated limewater into the filtrate at the ratio of limewater and filtrate equaled 1:30 (v/v), stirring the mixtures evenly and then let it standing until to jelly into Fairy Tofu.

Components analysis of Fairy Tofu

Water content

Water content was measured by drum wind drying oven (Nielsen, 2003).

Pectin content

Determination of pectin content followed inter-hydroxy-biphenyl colorimetry (Blumenkrantz and Asboe-Hansen, 1973).

Soluble sugar content

Soluble sugar content was determined by anthrone - sulfuric acid colorimetry (Zhang, 2009).

Protein content

Protein content was determined by the method of ultraviolet absorption (Zhang, 2009).

Cellulose content

Cellulose content was determined by gravimetric method (Zhang, 2009).

Vitamin C content

Vitamin C content was determined by 2,6-dichloroindophenol titrimetric method (Zhang, 2009).

The design for the preparation effect of Fairy Tofu by different metal salts

In the preparation process using fresh leaves of *P. puberula* to make Fairy Tofu, different metal salts, such as $CaCl_2$ solution (0.1%, 0.3% and 0.6%), $Al(NO_3)_3$ solution (0.1%, 0.3% and 0.6%) and $CuSO_4$ solution (0.1%, 0.3% and 0.6%) were respectively used to instead of saturated limewater. The experimental design was shown in TABLE 1.

Table. 1: Metal salts and concentration using Fairy Tofu preparation.

No.	Metal salt	Solution concentration/%
A	$CaCl_2$	0.1
B	$CaCl_2$	0.3
C	$CaCl_2$	0.6
D	$Al(NO_3)_3$	0.1
E	$Al(NO_3)_3$	0.3
F	$Al(NO_3)_3$	0.6
G	$CuSO_4$	0.1
H	$CuSO_4$	0.3
I	$CuSO_4$	0.6

The design of the treatment effect for the leaves and Fairy Tofu by enzymes

According to the design of TABLE 2, the fresh leaves of *P. puberular* were pretreated by different enzymes before making Fairy Tofu, involved 1% pectinase Y-23 (Japan), 1% cellulase (Japan) and 1% snailase (Solarbio, China). To cut the leaves into small square pieces of 0.25 cm^2, then placing the pieces of leaf into different enzyme solutions (the solid-liquid ratio was 1:20 w/v), respectively, to treat for 5 or 30 min at the temperature of 25℃, and with distilled water as controls. After soaking and filtering, to wash the pieces of leaf with distilled water so as to clear enzyme residues on the pieces. Next, to make Fairy Tofu by the traditional method. Meanwhile, using pectinase Y-23 to treat the leaves and Fairy Tofu in accordance with TABLE 3.

Table. 2: Using different enzymes to pretreat the leaves.

No.	Treatment solution	Treatment time/min
A	Distilled water	5
B	1% pectinase Y-23	5
C	1% cellulase	5
D	1% snailase	5
E	Distilled water	30
F	1% pectinase Y-23	30
G	1% cellulase	30
H	1% snailase	30

Table. 3: Pectinase treatment in the process of making Fairy Tofu.

No.	Treatment solution	Treatment method
A	Distilled water	Making Fairy Tofu after soaking the leaves for 2 h at 25℃
B	1% pectinase Y-23	Making Fairy Tofu after soaking the leaves for 2 h at 25℃
C	Distilled water	Following the traditional method to making Fairy Tofu
D	1% pectinase Y-23	Following the traditional method to making Fairy Tofu but replaced distilled water with pectinase solution
E	1% pectinase Y-23	Adding pectinase into Fairy Tofu after the homogenate jellied
F	1% pectinase Y-23	Adding pectinase into homogenate before Fairy Tofu formed

Extraction and dissolution for pectin in the leaves

Pectin extraction

According to the method described by Xu (2003) and Kratchanova (2004) to extract pectin in the leaves of *P. puberular*. Drying fresh leaves and making dry leaves powder, mixing dry leaves powder with HCl solution (pH=2, the solid-liquid ratio was 1:20 w/v). Heating the mixtures for 60 min at 90℃ with continuous stir. Using Buchner funnel to filter the hot mixtures and concentrating the filtrate to half of the total volume. Adding equal volume of anhydrous ethanol into the concentrated filtrate, stirring the mixtures evenly and then let it standing until to form jelly (pectin). Drying pectin at 30℃ drying oven and to obtain dry pectin powder.

Pectin dissolution

Mixing dry pectin powder with distilled water (the solid-liquid ratio was 1:40 w/v), stirring the mixtures until to pectin was dissolved. Injecting clear saturated limewater into the pectin solution at the ratio of limewater and pectin solution equaled 1:20 (v/v), stirring the mixtures evenly and then let it standing to form jelly.

RESULTS AND ANALYSIS

Effects of different metal salts for forming Fairy Tofu

In folk process to making Fairy Tofu, limewater or plant ashes were usually added into the homogenate which used the leaves of *P. puberula* to grind so that to accelerate forming the jelly. However, in this test, a new phenomenon was found, it was that the homogenate could be also jellied to form spontaneously Fairy Tofu if limewater or plant ashes were not added when making Fairy Tofu, and the appearance of this Fairy Tofu was more exquisite (FIGURE 1 A) to compare with control (FIGURE 1 B), but for a longer period of time to form jelly. So it could be considered that the limewater and plant ashes were not necessary in the formation of Fairy Tofu, their functions were supplying metal ions (e.g., Ca^{2+} and K^{+}) and adjusting pH to shorten the time of Fairy Tofu formation. Meanwhile, it was observed that divalent or trivalent metal ions (e.g., Ca^{2+}, Cu^{2+} and Al^{3+}) had the similar function for shortening the time of homogenate gelling, the effects to shorten time were Ca^{2+} shorter than Cu^{2+}, and Cu^{2+} shorter than Al^{3+}. On the other hand, the concentration of metal ions also influenced the yield and shape of Fairy Tofu (TABLE 4, FIGURE 2), the higher yield and smoother shape of Fairy Tofu could be obtained by Al^{3+}, but Al^{3+} is harmful to people's health and should not be taken in too much. Ca^{2+} and Cu^{2+} had no significant difference to the yield and shape of Fairy Tofu under the same ions concentration, but the higher concentration of divalent metal ions were, the lower yield and more irregular shape of Fairy Tofu had.

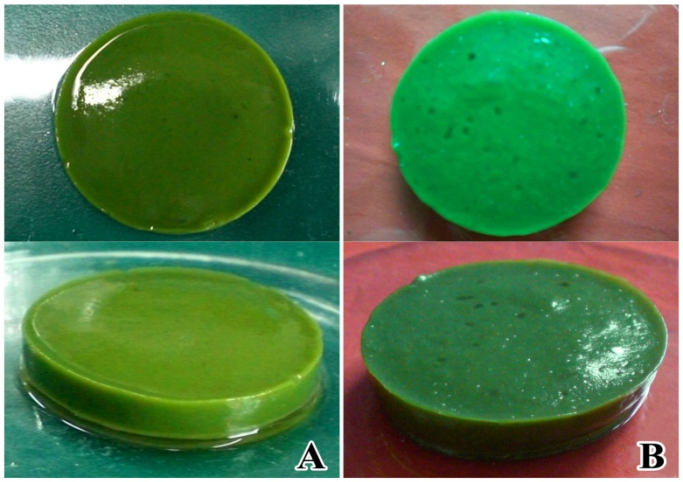

Fig. 1a,b: Fairy Tofu From Directly Leaf Homogenate (Not Adding Saturated Limewater), B) Fairy Tofu Made From The Traditional Method.

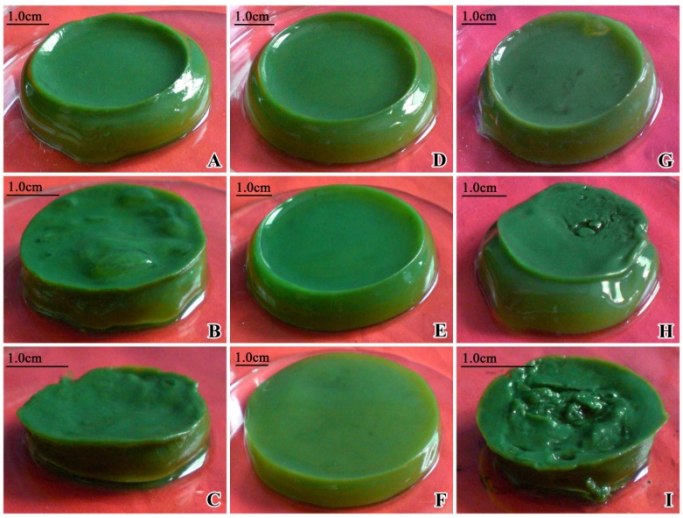

Fig. 2: Effect on production and texture of Fairy Tofu adding different metal salts into the leaves homogenate (the letters in the picture corresponding to the serial numbers in TABLE 1 and TABLE 4).

Table. 4: Effect of different metal salts for Fairy Tofu production. (Means of three replicates followed by the different letters indicate a significant difference between any two treatments, $p<0.05$).

No.	Metal salt	Production /g
A	0.1%$CaCl_2$	14.195c
B	0.3%$CaCl_2$	6.481d
C	0.6%$CaCl_2$	5.270de
D	0.1%$Al(NO_3)_3$	14.882bc
E	0.3%$Al(NO_3)_3$	16.895b
F	0.6%$Al(NO_3)_3$	19.504a
G	0.1%$CuSO_4$	15.732bc
H	0.3%$CuSO_4$	7.295d
I	0.6%$CuSO_4$	3.093e

Key component determination for forming Fairy Tofu

Basic components of Fairy Tofu

Adopting traditional method to make Fairy Tofu and analyzed its components in this test, the results showed that Fairy Tofu was mainly comprised of water, pectin, soluble sugars, proteins, etc. (TABLE 5). Besides water, pectin content was the highest in dry substance (about 30%). At the same time, using fresh leaves by heated water at 90℃ to soak for 60 min could be made Fairy Tofu (FIGURE 3 A), the difference of Fairy Tofu was just reflected on color to compare with traditional method, because chlorophyll was degraded, and the colors of lutein and carotene were presented. Dried leaves could be also made Fairy Tofu by above similar treatment (FIGURE 3 B). So, it could be determined that the basic components of Fairy Tofu were water-soluble substances.

Table. 5: The basic components of Fairy Tofu (Means of three replicates).

Assay index	Content/%
Water (Fresh weight)	98.52±0.31
Pectin (dry weight)	32.48±9.88
Soluble sugar (dry weight)	3.02±0.32
Protein (dry weight)	0.003±0.001

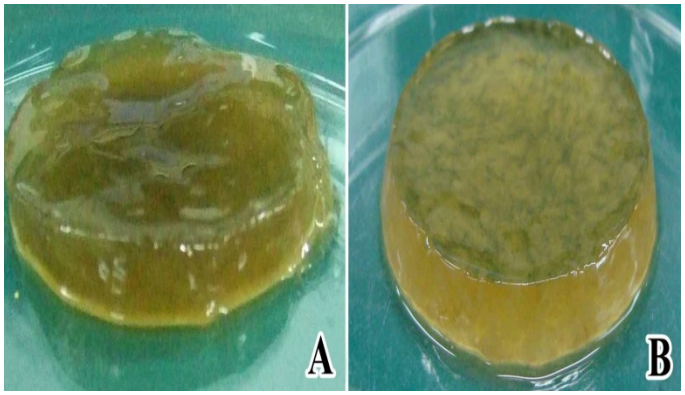

Fig. 3: Fairy Tofu comparison from fresh leaves and dried leaves by high temperature water bath.
A) Fairy Tofu made from fresh leaves by 90º C water bath for 60 min, B) Fairy Tofu made from dried leaves by 90℃ water bath for 60 min.

Key component of forming Fairy Tofu

The significant influence for forming Fairy Tofu was showed after using different enzymes to treat the leaves of *P. puberula* (FIGURE 4, FIGURE 5 and FIGURE 6). Fairy Tofu could be formed when the leaves were treated by pectinase Y-23 for short time (< 3 min), but its strength was lower (bearing capacity < 1 g weight. FIGURE 5 A) to compare with control (FIGURE 5 B). By distilled water treating as control (FIGURE 4 A and FIGURE 4 E), when the treated time by pectinase solution was prolonged (5 min to 30 min), Fairy Tofu was become loose structure and could be not shaped (FIGURE 4 B and FIGURE 4 F). And when the treated time was reached to 2 hours, Fairy Tofu could be not formed (FIGURE 6 B. FIGURE 6 A was control, namely with distilled water instead of enzyme solution).

Compared with the traditional method of making Fairy Tofu (FIGURE 6 C), it could not get Fairy Tofu that used pectinase solution to replace distilled water (FIGURE 6 D). As the conventional procedure of making Fairy Tofu, if adding certain concentration of pectinase into the filtrate after the filtrate jellied, the surface of Fairy Tofu was degraded (FIGURE 6 E). But if adding pectinase into the filtrate before the filtrate jellied, Fairy Tofu could be not formed (FIGURE 6 F). Furthermore, using cellulase to treat the leaves, Fairy Tofu would be not affected (FIGURE 4 C and FIGURE 4 G). Fairy Tofu could be formed by snailase (a mixture both pectinase and cellulose) to treat the leaves for short time (< 3 min), but its texture was relatively loose (FIGURE 4 D). Prolonged the time of snailase treatment (> 5 min), Fairy Tofu could be not formed (FIGURE 4 H). So, pectin was a vital component for forming Fairy Tofu (FIGURE 7).

To synthesize the results both basic components of Fairy Tofu and enzymes treatment, a conclusion would be drawn that the key component to form Fairy Tofu was pectin.

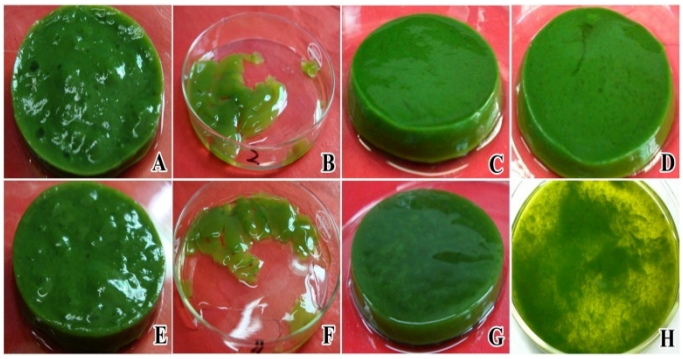

Fig. 4: Effect on Fairy Tofu formation using enzymes to pretreat the leaves (the letters in the picture corresponding to the serial numbers in TABLE 2).

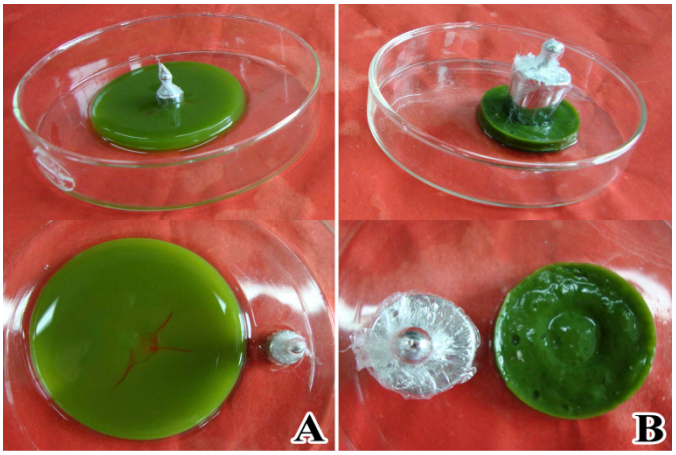

Fig. 5: Jelly strength's change of Fairy Tofu by pectinase to pretreat the leaves in short time. A) Fairy Tofu made from the leaves by pectinase to pretreat for 3 min, B) Fairy Tofu made by the traditional method.

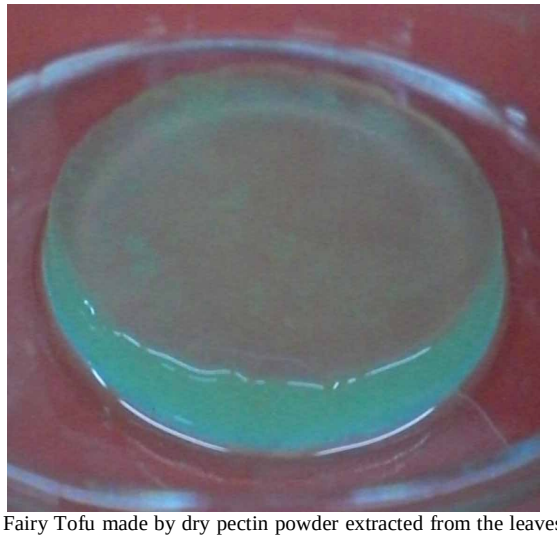

Fig. 7: Fairy Tofu made by dry pectin powder extracted from the leaves of *P. puberula.*

DISCUSSION

Fairy Tofu was formed by a gelatum in the leaves of *P. puberula*, this study showed that the gelatum was pectin, which was consistent with the results of Li (1988) and Luo (1999). They thought that main component of Fairy Tofu was pectic substances rather than proteins, the jelly (Fairy Tofu) would be formed when pectic substances were more than 8.8%. Pectin was mainly present in plant cell walls and could stick adjacent cells together. Moreover, pectin had gel's excellent properties; it was an important additive to be used in food industry (Willats et al., 2006; Mohnen, 2008; Hu et al., 2006). It has been reported that pectin's degree of esterification in *P. puberular* leaves was about 76% (Ma, 2007), namely a high-ester pectin. The high-ester pectin could self-gel under the conditions of pH < 4 and higher soluble solids, but low-ester pectin was able to form a gel network structure in the presence of divalent or trivalent metal ions such as Ca^{2+} and Al^{3+} (Ström et al., 2007). In this test, the homogenate (pH 5.8) which was ground from the eaves of *P. puberular* could self-gel slowly to form Fairy Tofu, the gelling process would be accelerated if adding the divalent or trivalent metal ions into the homogenate. So, it needs to be further studied whether the metal ions and the high-ester pectin in the leaves linked as Egg-box Model (Fennem, 1996; Ström et al., 2007) to accelerate gelling or the high-ester pectin and the low-ester pectin co-existed in the leaves of *P. puberula*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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