

Biostudy on Traditional Chinese Medicine Massa Medicata Fermentata

Frank Qiang Fu, Mingshu Xu,* Zhijiang Wei, and Weidong Li*



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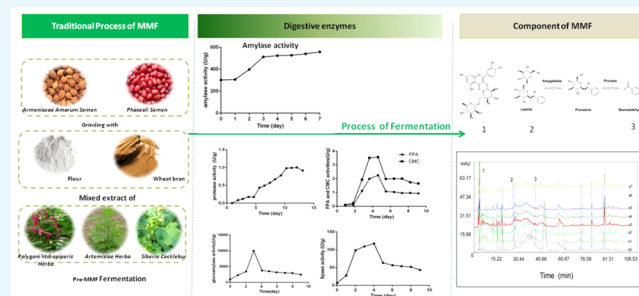
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ABSTRACT: Massa Medicata Fermentata (MMF) has been used for a long time by the Chinese. MMF is used widely in feed additives and human medicinal applications throughout the world; however, there have only been a few reports about the biostudy of its fermentation mechanism and medicinal ingredients. To safely use MMF, we observed the changes in the ingredients and amylase activity for several raw materials during the fermentation process of MMF. We are going to explore the basis of pharmacodynamic substances and the purpose of MMF to provide support for safe use in clinics. This biostudy data demonstrated that the ingredients such as amygdalin, benzaldehyde, and rutin were gradually degraded during the process of fermentation, and the fermented MMF did not contain amygdalin and benzaldehyde. The HPLC fingerprint of fermented MMF for 7 days is similar to the chemical composition of the original unfermented MMF with a similarity of only 0.106. Meanwhile, the activities of amylase in fermented MMF had gradually increased, and the content of organic acids also had increased. According to our biostudy, we found that the raw material chemical composition of MMF in the process of fermentation was affected by microorganisms and various substances. The conclusions of our study determined that the initial components of MMF are not identical to the pharmacodynamic components. We also conclude that amylase activity explains the pharmacological activity of MMF to a certain extent, but it is likely not the only factor. The implication not only provides the initial knowledge of MMF but also implies the further exploration of this popular traditional medicine.



INTRODUCTION

During the COVID-19 outbreak throughout the world, traditional Chinese medicine (TCM) has been proven to stop mild to severe symptoms of COVID-19 in China,^{1,2} and there is lab evidence that TCM can be used safely to fight the epidemic.^{1,2} Many traditional foods and traditional Chinese medicines are made from fermentation, such as wine, sauce, and Massa Medicata Fermentata (MMF). During the fermentation process of the foods or drugs, the influence of microorganisms is an important step in the production; for example, senna glycoside is degraded into diarrhea-causing emodin under the influence of microorganisms in our human bacterial gut system.³ For use as a purgative drug, the activity of microbial degradation of *Scutellariae Radix* is higher than that of the root.⁴

Since the beginning of the third century, it has been well-known that MMF is a fermented drug used in China, and now it is very popular in research on the gut microbiota in the world as the intestinal microenvironment impacts the lungs by the gut–lung axis and the brain by the gut–brain axis. We already understand that the gut microbiota are responsible for the health of individuals starting from birth and during early life, adulthood, and aging. MMF is used as a digestive agent where it functions in strengthening the gut and spleen and in

balancing the digestive system. In addition, Massa Medicata Fermentata is used for gastroenteritis and dyspepsia with low gastric acidity.⁵ MMF is a fermented drug that has been widely used since the beginning of the third century. MMF is used as a digestive agent clinically, and it has the functions of strengthening the spleen and gut and balancing the digestion system.⁶ MMF is used for dyspepsia and gastroenteritis with low gastric acidity. MMF is a traditional Chinese medicine with natural fermentation, and MMF is made by *Artemisia annua*, *Polygonum hydropiper*, *Xanthium sibiricum*, red bean, bitter almond, flour, and wheat bran⁷ (National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China (3 Part) [S], 2012). Modern studies have shown that the fermented MMF can improve the disorder of the bacterial system in the gut, and the unfermented MMF does not have these pharmacological activities. According to “The People’s

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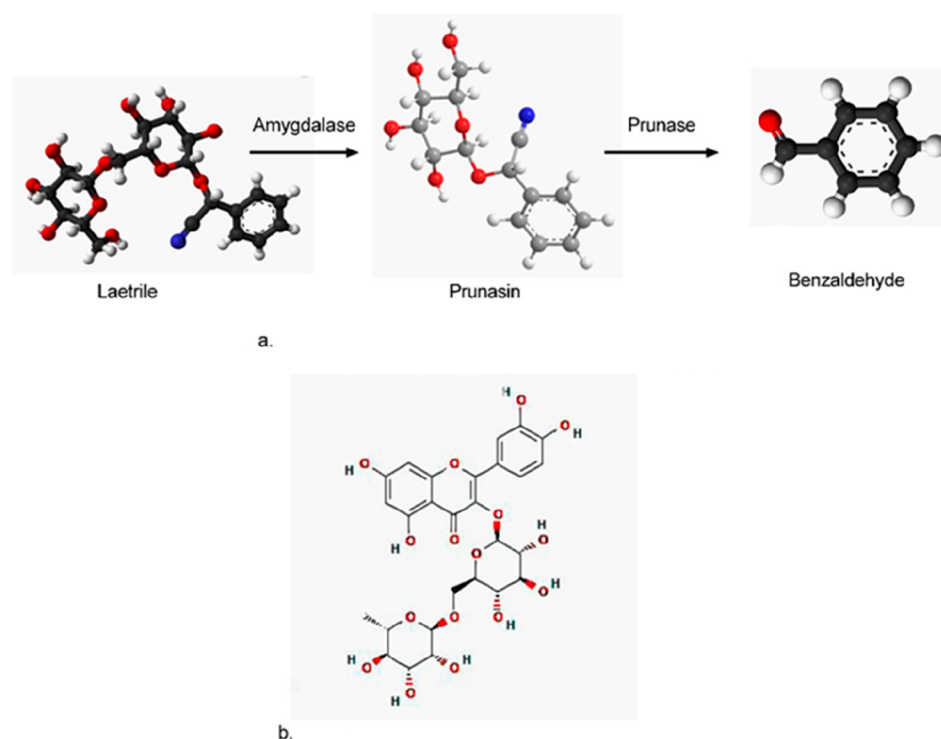


Figure 1. (a) Chemical structures of laetrile and benzaldehyde (in the presence of water laetrile degraded by amygdalase and prunase). (b) The chemical structure of rutin.

Republic of China Pharmacopoeia 2010”, in a set prescription of fermented herbal medicine, MMF can account for up to 47%. Although MMF has been used for a long time by the Chinese and there is also a huge domestic market today, there are few studies about the fermentation and metabolic processes, and its medicinal ingredients have not been determined. This is resulting in poor controllability of the production and the market.⁸ To ensure the safety and effectiveness of MMF for clinical use, we have to study the MMF fermented process and finally to explore the active medicinal ingredients of MMF.

We know that MMF is made by *Artemisia annua*, *Polygonum hydropiper*, *Xanthium sibiricum*, red bean, bitter almond, flour, and wheat bran (National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China (3 Part) [S], 2012) (shown in Figure 1). As shown in Table 1, MMF raw materials contain various ingredients, including cellulose, hemicellulose, starch, protein, volatile oil, fat, and other ingredients. *Xanthium sibiricum* and other volatile oils disappeared during the production process.^{9,10}

Table 1. MMF Main Components of the Crude Drugs

wheat bran	cellulose, hemicellulose, lignin, protein, vitamins
flour	starch, protein (amylase, protease, lipoxigenase, polyphenol oxidase, and peroxidase); pentosan; vitamin
<i>Phaseoli semen</i> (PS)	protein, starch, fat, saccharides
<i>Siberia cocklebur</i> (SC)	volatile oil, fatty oil, sesquiterpene lactones
<i>Artemisiae herba</i> (AH)	volatile oil, sesquiterpenes (artemisinin), flavonoids, coumarin
<i>Artemisiae amarum semen</i> (AAS)	glycosides (laetrile); protein (amygdalase, prunase), fat
<i>Polygoni hydropiperis herba</i> (PHH)	volatile oil, flavonoids (rutin)

Artemisinin of *Artemisia annua* is a common medicinal ingredient.¹¹ It has been found that artemisinin is degraded, and octadecenoic acid of the organic acid substances is detected during the fermentation of MMF.¹² MMF is clinically used as a digestive aid and may show acidity. This study explores the change in acidity during the fermentation process of MMF.

Bitter almond has protein ingredients such as Amygdalin and Prunase.^{13–15} As shown in Figure 1, Amygdalin is one of the main medicinal ingredients in bitter almond, and it would be degraded to produce benzaldehyde to undergo enzymatics in the presence of water. Benzaldehyde and amygdalin are two kinds of medicinal ingredients in bitter almond, and these two ingredients are representative medicinal ingredient for almonds. This study observed changes in the content of amygdalin and benzaldehyde during fermentation.

As shown in Figure 1, AAS contains amygdalase, prunase, and other protein ingredients^{7,8} as the main pharmacological ingredients. For laetrile, when it is in water, enzyme degradation occurs to produce benzaldehyde. Thus, laetrile and benzaldehyde are two kinds of major ingredients in AAS. Our study observed the changes of content for laetrile and benzaldehyde during the MMF fermentation process. PHH contains volatile oils and flavonoids, and the main pharmacological ingredient is rutin (Figure 3) in PHH. In the biostudy, we observed the changes of rutin's content during the MMF fermentation process. In the experiments, we observed the changes of the HPLC fingerprint for MMF during the fermentation process, so we could explore the changes of ingredients and the ratio of ingredients for MMF.

Recently, researchers isolated a large amount of yeast and other bacteria and a small amount of lactic acid bacteria from MMF.^{16,17} Some researchers suggest that enzyme activities can be used as quality standards.^{18,19} MMF uses the process of stir-

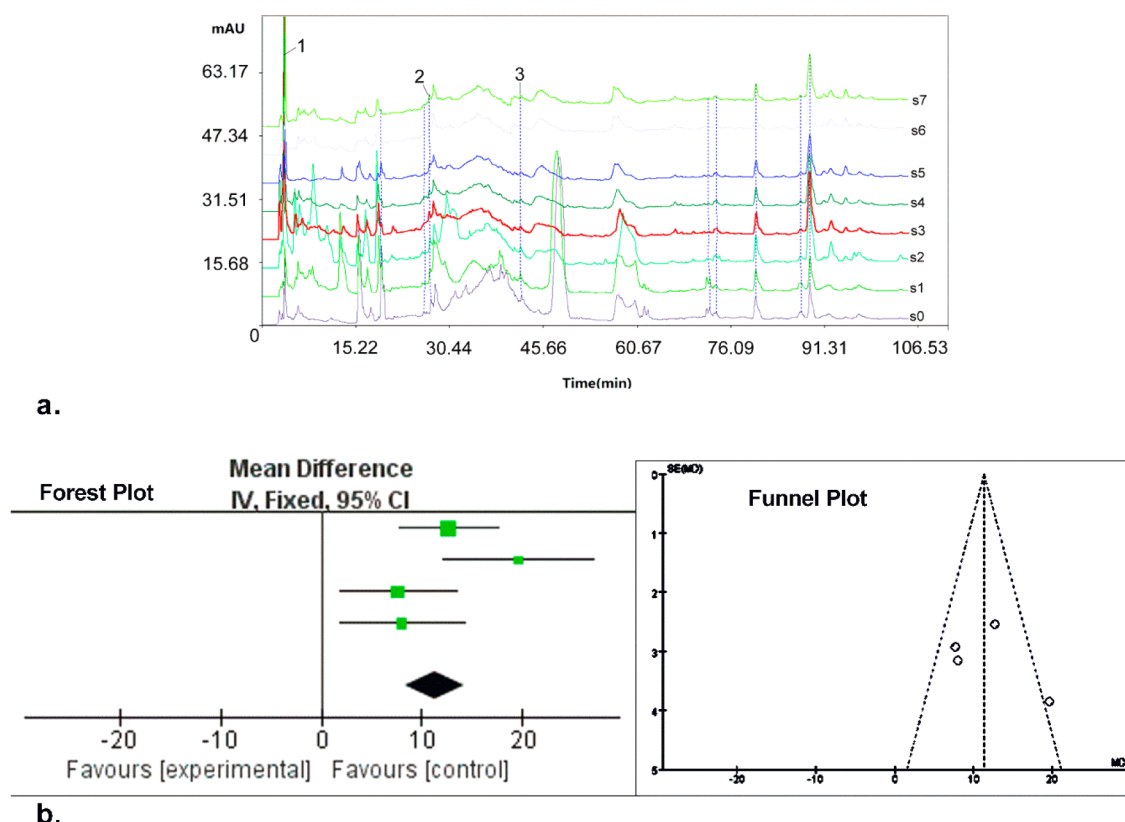


Figure 2. (a) Fingerprint of MMF with increasing fermenting time. 1, rutin; 2, laetrile; 3, venzaldehyde; s0–0 day, s1–1 day, s2–2 day, s3–3 day, s4–4 day, s5–5 day, s6–6 day, and s7–7 day. (b) Comparison: propulsion in the small intestine between used MMF and unused MMF for mice. Heterogeneity: $\chi^2 = 7.61$; $df = 3$ ($P = 0.05$); $I^2 = 61\%$. Test for overall effect: $Z = 7.51$ ($P < 0.00001$).

Table 2. Massa Medicata Fermentata Similarity in Increasing Fermenting Time (mAU)

	S0	S1	S2	S3	S4	S5	S6	S7	control
S0	1	0.943	0.056	0.091	0.071	0.155	0.163	0.106	0.122
S1	0.943	1	0.103	0.126	0.108	0.174	0.109	0.169	0.182
S2	0.056	0.103	1	0.525	0.409	0.352	0.084	0.453	0.4
S3	0.091	0.126	0.525	1	0.715	0.494	0.301	0.51	0.62
S4	0.071	0.108	0.409	0.715	1	0.487	0.407	0.502	0.631
S5	0.155	0.174	0.352	0.494	0.487	1	0.357	0.838	0.848
S6	0.163	0.109	0.084	0.301	0.407	0.357	1	0.204	0.339
S7	0.106	0.169	0.453	0.51	0.502	0.838	0.204	1	0.791
control	0.122	0.182	0.4	0.62	0.631	0.848	0.339	0.791	1

frying until yellow and stir-baking to brown. Stir-frying until yellow can keep up to 60% efficacy of crude materials, while stir-baking to brown causes total loss of enzyme efficacy. Some studies have shown that fry-processed MMF loses almost all the activities of amylase and proteinase.²⁰ Some researchers have already suggested that unfermented MMF could be used.²¹ In these cases, we observed changes of amylase activity during its fermented process. In the biostudy, we systematically analyzed the main chemical ingredients of crude drugs from MMF including the amylase activities and acidity. We to explore the new ingredients with conducive for the digestion and to provide the scientific data for the safety used of clinical drug.

RESULTS AND DISCUSSION

HPLC Fingerprint. High-performance liquid chromatography is a technique used in *China Pharmacopoeia* to separate, identify, and quantify each component for our MMF. The software has been used in processing, and the results are shown in Figure 2a and Table 2. We observed a variety of components in traditional Chinese medicine (TCM)^{9,21} at the same time.^{20–23} We studied the HPLC fingerprint of the MMF of 0–7 days fermentation samples and obtained HPLC fingerprint study data (according to the national pharmacopoeia committee “Chinese medicine chromatographic fingerprint similarity evaluation system” 2015 software for processing), and the results are shown in Figure 2a and Table 2.

As shown in Figure 2a and Table 2, MMF extracts were analyzed by HPLC. The fingerprint similarity was 0.943 between day 0 and day 1; 0.103 between day 1 and day 2;

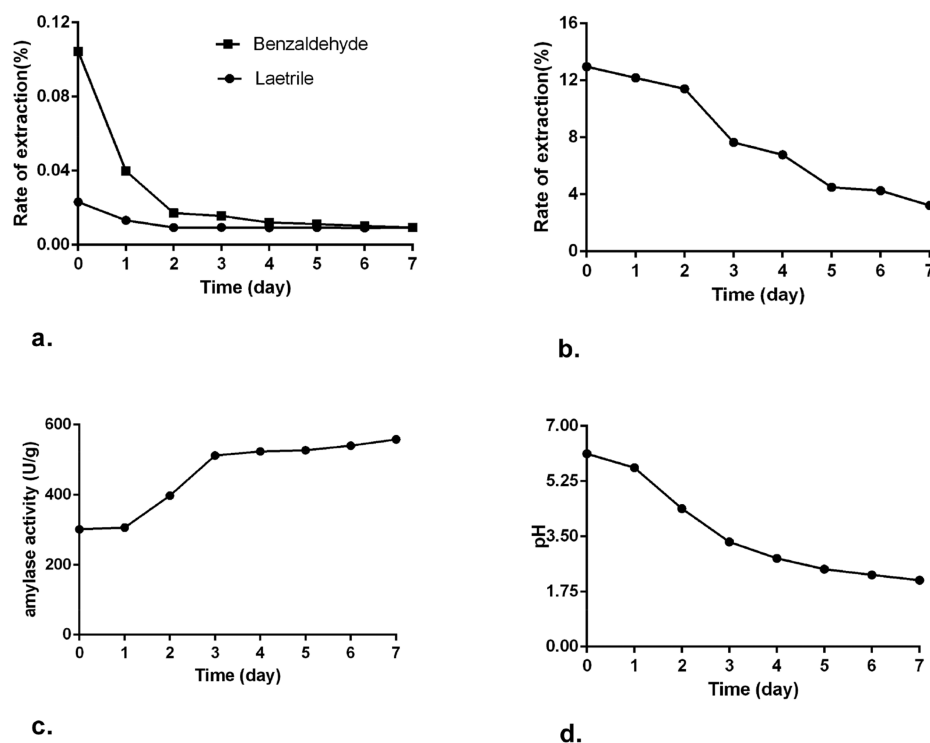


Figure 3. (a) Benzaldehyde and laetrile contents with decreasing fermentation time. (b) The rutin content with decreasing fermentation time. (c) The amylase activity with increasing fermentation time. (d) pH with decreasing fermentation time.

0.525 between day 2 and day 3; 0.715 between day 3 and day 4; 0.487 between day 4 and day 5; 0.387 between day 5 and day 6; and 0.204 between day 6 and day 7. The HPLC fingerprints showed dynamic changes during fermentation, which means that the components and the ratio between components were changing as well. The most significant changes happened during day 1 to day 2. Next, significant changes happened between day 6 to day 7. The fingerprint similarity was only 0.122 between day 0 and day 7. In Figure 2a, peak 1 was rutin; peak 2 was laetrile; and peak 3 was benzaldehyde, and these components were degraded to small molecules by micro-organisms during fermentation. In this study, we found that changes of components of the crude drugs from MMF are significant; therefore, the main components of crude drugs are not the pharmacodynamic components of MMF. In Figure 2a and Table 2, *Massa Medicata Fermentata* extracts were analyzed by HPLC.^{22,23}

MMF Impacts Propulsion in the Small Intestine. Recent advances in microbiota explorations have led to improved knowledge of the communities of commensal microorganisms within the human body.^{24,25} We aimed to analyze how MMF influences the propulsion in the small intestine and used the review-manager software 5.3 to analyze the propulsion in the small intestine of MMF using Chinese published literature about MMF,^{26–28} and there is a significant difference in the propulsion in the small intestine between used MMF mice and unused MMF mice (heterogeneity: $\chi^2 = 7.61$; $df = 3$ ($P = 0.05$); $I^2 = 61\%$; test for overall effect: $Z = 7.51$ ($P < 0.00001$), Figure 2b). When the I^2 value exceeds 25%, 50%, and 75%, it indicates that our study has low, moderate, and high heterogeneity. It is generally believed that $I^2 > 50$ has substantial heterogeneity. For the Cochrane Q test, the essence is the chi-square test, and the method of qualitative analysis of heterogeneity, $P > 0.1$, means no statistical

heterogeneity between our studies; on the contrary, $P < 0.1$ means there is heterogeneity between used MMF and unused MMF (Figure 2b).

Used MMF digestive liquid catalyzes the breakdown of food into many small molecules which can be absorbed and utilized by our gut and which can be further absorbed into our body for lung respiration.^{29,30} According to the gut–lung axis, used MMF treatments can impact the intestinal microenvironment and impact metabolic functions as well as immune responses in our body.^{29–32} The gut–lung axis has emerged as a specific axis with intensive dialogues between the gut and lungs, involving each compartment in a two-way manner, with both microbial and immune interactions.^{31,32} The metabolites produced by used MMF treatments not only modulate gastrointestinal immunity but also impact distal organs like the lungs and brain.^{29–32}

Benzaldehyde and Laetrile. Benzaldehyde (C_6H_5CHO) is an organic compound consisting of a benzene ring with a formyl substituent, and it is the simplest aromatic aldehyde. We analyzed benzaldehyde and laetrile content by our employed HPLC. The analysis showed laetrile, and benzaldehyde levels decreased sharply from day one (Figure 3a). Laetrile is a compound that has been used as a treatment for people with cancer, and laetrile is another name for amygdalin. Amygdalin is a bitter substance found in fruit pits, such as apricots, raw nuts, lima beans, clover, and sorghum.

The method to analyze laetrile and benzaldehyde was established by HPLC, and the content of laetrile and benzaldehyde was measured. As shown in Figure 3a, the analysis showed that laetrile and benzaldehyde content decreased sharply from day 1. Laetrile was not detectable from day 2. Benzaldehyde was undetectable from day 7. Part of laetrile was degraded by amygdalase and prunase. Some laetrile and benzaldehyde were degraded by other microbials during

the fermentation process since laetrile and benzaldehyde are unstable and degraded easily. Laetrile and benzaldehyde are not the pharmacodynamic ingredients of MMF.

Rutin. Rutin is a bioflavonoid, or plant pigment, that is found in certain vegetables and fruits. Our sample rutin standard mixture was added to flour and fermented, and this increased the initial rutin content so that the rutin became easy to detect. Standard rutin mixed with flour was fermented. This increased the initial rutin content so that rutin is easy to detect. As shown in Figure 3b, rutin content gradually decreased with fermentation time. The content had fallen significantly since days 2–3 and then decreased gradually. Rutin might be degraded by microbes in flour or other materials. We concluded that rutin is not one of the pharmacodynamic components of MMF.

Amylase Activity. Amylase is an enzyme that catalyzes the hydrolysis of starch into sugars, and amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. We tested the amylase activity of Massa Medicata Fermentata (Figure 3c) in different fermenting times and showed that there was no difference between day 0 and day 1, but it was significantly different between day 0 and other days. Amylase activity in the second fermenting day also differed significantly from the third day to the seventh day. However, there was no significant difference in the amylase activity between samples of 3 and 7 days. Amylase activity was increased slowly and gradually reached a plateau region after the third day. Amylase activity which was increased during the fermentation process not only degraded components of crude drugs from MMF but also could impact the digestion of foods in the human gut.

Acidity. As shown in Figure 3d, the acidity of MMF in different fermenting times showed that there was a significant difference between day 0 and the third day, and for the acidity change, there was no significant difference between days 4 and 7. There was a plateau as the time passed, and then the acidity of MMF increased. This is because it produces abundant organic acid materials during fermentation. For example, aliphatic from AAS was degraded to 95% content of unsaturated fatty acid.^{24,25} Acidic materials could reduce the pH of the human gut and impact the secretion of the pancreas, and then it could be used as a digestive.^{35,36}

In the fermentation process of MMF, it has two tendencies: first is that the main components of crude drugs from MMF degraded to small molecules by some enzyme, and the acidity became higher; second is that the amylase activity increased. The fermentation process of MMF includes the interaction between components of crude drugs and micro-organisms, and in this process it produced some materials which are digestive.

As shown in Table 3, wheat bran content in MMF is the highest and reaches 42%. Wheat bran contains about 62% hemicellulose, 24% cellulose, and 11% lignin. These indigestible wheat bran fibers could absorb water to promote intestinal peristalsis and make it easy to defecate by intenerating excrements. Vitamin B1, which is part of the digestive product of wheat bran, could regulate carbohydrate metabolism and promote digestion.^{34–36} Flour and PS contain proteins,^{37,38} and they could be degraded to some essential amino acids for the human body and provide nutrients that are easy to absorb in the human body. These pharmacological effects are in accord with the clinical effect of traditional Chinese medicine, which is digestive and not suitable for hyperacidity indigestion.

Table 3. Fermented Product of Crude Drugs from Massa Medicata Fermentata

raw material of drugs	proportion of MMF	product of fermentation
wheat bran	42%	wheat bran fiber vitamin B1
flour	21%	saccharides, amino acid
<i>Phaseoli semen</i> (PS)	0.8%	amino acid
<i>Siberia cocklebur</i> (SC)	1.3%	volatile oil was volatilized
<i>Artemisiae herba</i> (AH)	1.3%	octadecanoic acid
<i>Armeniacaeamarum semen</i> (AAS)	0.8%	hydrocyanic acid was volatilized, amino acid
<i>Polygonihtdropiperis herba</i> (PHH)	1.3%	volatile oil was volatilized

In this study, we showed that the original medical components were not pharmacodynamic components, and amylase activity could explain some pharmacological activity of MMF to some extent but could not explain it completely. Frequently, MMF uses the process of stir-frying until yellow or stir-baking to brown, which causes total loss of enzyme efficacy.²⁰ The digestive contents diluted hydrochloric acid and pepsase, pancreatin, amylase, etc.;³³ likewise, MMF as a digestive contents abundant microbial enzymes and a certain acidity and wheat bran fiber which promote intestines peristalsis. MMF has been widely used in China for a long time, but there are no studies on its chemical components and mechanism. This study started to make the exploration of the system.

CONCLUSION

This biostudy has provided initial knowledge of Massa Medicata Fermentata, and the results imply that deep exploration of this popular traditional medicine should be done to further focus on outbreaks of new human diseases worldwide. Massa Medicata Fermentata acts as a digestive and relies on abundant microbial enzymes, particularly acidities, and wheat bran fiber, which promotea intestinal peristalsis. The present digestive contents in Massa Medicata Fermentata include diluted hydrochloric acid, pepsin, pancreatic, and amylase. The process of stir-frying and -baking Massa Medicata Fermentata results in a total loss of enzyme efficacy. Although Massa Medicata Fermentata is widely used in China, there is a lack of studies regarding its chemical components and fermentation mechanisms. Our biostudy determined that the initial components of Massa Medicata Fermentata are not identical to the pharmacodynamic components. We also conclude that amylase activity explains the pharmacological activity of Massa Medicata Fermentata to a certain extent, but it is likely not the only factor.

MATERIALS AND METHODS

Materials. Acetonitrile (HPLC grade), distilled water, flour, methanol (analytical grade), laetrile, benzaldehyde, and rutin were purchased from China National Institutes for food and drug. AAS, PS, AH, PHH, SC, and wheat bran were purchased from Changan Angguo Pharmacy Company, China.

Methods. *Preparation of the Test Sample of Flour and Fermentated AAS Product.* According to the National Chinese medicine preparation, samples were prepared by using 2500 g of flour and 100 g of AAS. AAS was smashed and filtered through a 20-mesh filter and mixed with flour. About 2000 mL of warm water was added to leaven gradually. The mixture was rubbed to a round shape. The standard is that the

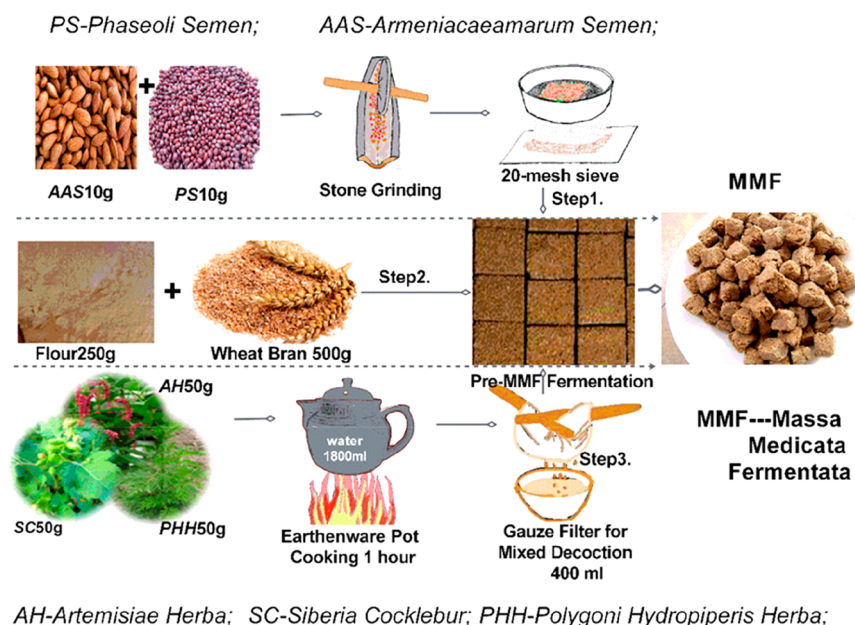


Figure 4. Preparation of Massa Medicata Fermentata

mixture will be chunky when grabbed in the hand, and it will disperse when thrown at the table. The mixture was finally put in a mold and fermented for 7 days in a constant temperature and constant humidity oven. One small chunk was taken out every day and dried in an air-dry oven at 40 °C.

Preparation of the Test Sample of Flour and Rutin Fermented Product. According to the National Chinese medicine preparation, samples were first prepared by dissolving 64.6 mg of rutin standard in about 150 mL of warm water. The solution was added into 400 g of flour gradually and mixed well. The mixture was rubbed to a round shape. The standard is that the mixture will be chunky when grabbed in the hand, and it will disperse when thrown at the table. The mixture was finally put in a mold and fermented for 7 days in a constant temperature and constant humidity oven. One small chunk of leaven was taken out every day and dried in an air-dry oven at 40 °C.

Preparation of the Test Sample of MMF Fermented Product. According to the National Chinese medicine preparation, 500 g of wheat bran, 10 g of AAS, and 10 g of PS were smashed and mesh screened individually, followed by mixing with 250 g of flour. Amounts of 50 g of AH, 50 g of PHH, and 50 g of SC were mixed with 1800 mL of distilled water and boiled for 1 h, filtered, and concentrated to 400 mL. The hot extract was added to the above well-mixed powder and rubbed to a chunky shape. The mixture was placed in molds in a 30 °C, 85% humidity constant temperature and constant humidity oven. One small chunk of MMF was taken out every day for a consecutive 7 days and dried in a 40 °C air-drying oven (Figure 4).

Preparation of the HPLC Test Sample. AAS and Flour Sample. An amount of 30 g of AAS and a flour sample were taken out every day, mixed with 100 mL of methanol, sealed, and extracted by an ultrasound. The extract was filtered and used for laetrile and benzaldehyde detection by HPLC. Each sample was repeated three times. Laetrile control sample: weight of 6.05 mg of laetrile and put into a 10 mL volumetric flask, add methanol, and dissolve laetrile by ultrasound to make 0.605 mg/mL of a control mother liquid, which was filtered

through a 0.45 μ m filter and used as a control sample. Benzaldehyde control sample: weight of 103.08 mg of benzaldehyde and put into a 100 mL volumetric flask and add methanol to make 1.0308 mg/mL of control mother liquid. The control sample was filtered by a 0.45 μ m filter and used as a control solution.

Rutin and Flour Sample. An amount of 2.00 g of rutin and a flour sample were taken out every day, mixed with 50 mL of methanol, sealed, and extracted by an ultrasound for 30 min. The extract was filtered, and 10 mL of filtered extract was dried by evaporation and dissolved in 5 mL of methanol in a volumetric flask and then used for the HPLC test sample. Each sample was repeated three times. Rutin control sample: 2.29 mg of rutin was weighed and put into a 10 mL volumetric flask and dissolved by methanol in an ultrasound to make 229 μ g/mL of control mother liquid. An amount of 1 mL of mother liquid was brought to 10 mL by adding methanol and then the concentration was 22.9 μ g/mL. It was filtered through a 0.45 μ m filter and used as a control sample.

MMF HPLC Fingerprint Test Sample. Taking MMF samples from days 0–7, respectively, each 15 g, precisely add 100 mL of methanol. The bottle was sealed and weighed at 25°. The extraction lasted for a half hour, and then it was cooled and weighed again. The weight loss was made up by adding methanol. The mixture was mixed well and filtered. An amount of 20 mL of filtrate was taken and evaporated to dry and transferred to a 5 mL volumetric flask. The extract was mixed well and filtered through a 0.22 μ m filter, and then it was ready for HPLC assay. Each sample was repeated three times.

Detection Method of HPLC. AAS and Flour Sample. A Waters 1525 HPLC and a Diamonsil C18 chromatographic column were used. The mobile phase was A: acetonitrile, B: water. Acetonitrile–water gradient elution: 0–5 min, 5%:95%; 5–15 min, 5% \rightarrow 10%:95% \rightarrow 90%; 15–25 min, 10% \rightarrow 25%:90% \rightarrow 75%; 25–45 min, 45% \rightarrow 55%:55% \rightarrow 45%. Detection wavelength: 0–30 min: 207 nm, 30–45 min: 249 nm. Flow velocity: 1 mL/min. Column temperature: 30 °C. Injection volume: 10 μ L. Laetrile standard curve: $y = 1000000x + 140309$, $R = 0.9995$, linear range:

0.1815~6.0500 μg . Benzaldehyde standard curve: $y = 7000000x + 3308.9$, $R = 0.9995$. Linear range: 0.0015~0.1532 μg . Detection procession, stability, recovery, and repeatability all satisfy the requirements.

Rutin and Flour Sample. A Diamonsil C18 chromatographic column was used. The mobile phase was A: acetonitrile, B: water. Acetonitrile–water gradient elution: 0~10 min, 20% \rightarrow 30%:80% \rightarrow 70%; 10~15 min, 30% \rightarrow 100%:70% \rightarrow 0%; 15~25 min, 100%:0%; 25~30 min, 100% \rightarrow 20%:0% \rightarrow 80%. Detection wavelength: 360 nm. Flow velocity: 1 mL/min. Column temperature was 30 °C. Injection volume was 10 μL . Rutin standard curve: $y = 1000000x - 3144.3$, $R = 0.9999$, linear range: 0.01145 ~0.1374 μg . Detection procession, stability, recovery, and repeatability all satisfy the requirements.

MMF HPLC Fingerprint. We used a Diamonsil C18 chromatographic column. The mobile phase: A: acetonitrile, B: water. Acetonitrile–water gradient elution: 0~10 min 2%:98%; 10~12 min, 2%:98%; 12~15 min, 2% \rightarrow 5%:98% \rightarrow 95%; 15~30 min, 5% \rightarrow 24%:95% \rightarrow 76%; 30~40 min, 24% \rightarrow 60%:76% \rightarrow 40%; 40~55 min, 60% \rightarrow 75%:40% \rightarrow 25%; 55~70 min, 75% \rightarrow 94%:25% \rightarrow 6%; 70~84 min, 94%:6%; 84~85 min, 94% \rightarrow 100%:6% \rightarrow 0%; 85~105 min 100%:0%. Detection wavelength was 280 nm. Flow velocity: 0.5 mL/min 0~10 min, 1 mL/min 10~115 min. Column temperature was 30 °C. Injection volume was 10 μL . This assay, the methodology test precision, repeatability, and stability are good, and the RSD is less than 3%. The sample recovery rate of the detection range between 95%~105% meets the requirements of test accuracy.

Amylase Detection Enzyme Substrate Buffer Preparation. Amounts of 9.0 g of sodium chloride, 22.6 g of anhydrous disodium hydrogen phosphate, and 12.5 g of anhydrous potassium dihydrogen phosphate were dissolved in approximately 500 mL of distilled water by boiling. An amount of 0.4 g of soluble starch was mixed with 10 mL of distilled water, and the suspension was transferred to the above boiling solution. The mixture was cooled to room temperature. An amount of 5 mL of 37% formaldehyde solution was added to the mixture, and the volume was brought to 1000 mL by distilled water. This was the buffer solution of pH 7.0 and 0.4 g/L of enzyme substrate. Iodine storage solution (0.1 mol/L): 1.7835 g of potassium iodate and 22.5 g of potassium iodide were dissolved in distilled water. An amount of 4.5 mL of hydrochloric acid was added, and the volume was brought up to 500 mL. After thorough incorporation, the final solution was stored in a brown bottle at 4 °C. Diluted iodine solution (0.1 mol/L): Iodine storage solution was diluted ten times and kept in a brown bottle and used fresh. Enzyme solution extraction: 5.0 g of MMF after 0~7 days fermentation was smashed and filtered by a 20-mesh filter. An amount of 50 mL of distilled water was added and stirred for 30 min in a 40 °C water bath. Fully dissolve the enzyme protein by filtration, and put the filtrate aside. The reaction was prepared by mixing 1.0 mL of substrate buffer and 0.2 mL of enzyme extract mixed in 40 °C water for 7.5 min, and 1.0 mL of diluted iodine solution was added to 6.0 mL of distilled water. The spectrophotometer was blanked using distilled water, and the absorbance was read at 660 nm. Each sample was repeated three times. At the same time, we did not add the enzyme-controlled experiment. Activity = (AB - AU)/AB \times 800. AB: blank absorbance; AU test tube absorbance. Unit Definition: One unit is defined as

the amount of enzyme required to hydrolyze 10 mg of starch in 30 min at 40 °C in a total reaction volume of 100 mL.

pH Detection. We weighed 1.0 g of MMF after 0~7 days fermentation, smashed and filtered by a 20-mesh filter, and dissolved it in 10 mL of water in a volumetric flask and stirred it for 30 min. We used it for a pH test sample and used a pH meter (Shanghai Sanxin instrument and meter plant) testing solution to get the pH value. We repeated it 3 times.

AUTHOR INFORMATION

Corresponding Author

Weidong Li – School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 102488, People's Republic of China; Email: liweidong2005@126.com

Authors

Frank Qiang Fu – School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 102488, People's Republic of China; orcid.org/0000-0002-5854-3564

Mingshu Xu – College of Marine Science, Shandong University at Weihai, Weihai 264209, People's Republic of China

Zhijiang Wei – School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 102488, People's Republic of China

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acsomega.0c00816>

Author Contributions

*M.X. contributed equally to this work. Frank Qiang Fu: Conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; and wrote the paper. Mingshu Xu: Conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; and contributed reagents, materials, analysis tools, or data. Weidong Li: he is the supervisor of Ph.D. at Beijing University of Chinese Medicine. Zhijiang Wei: postgraduate student in the University, and he did the assistant job.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

PHH, Polygonihdropiperis Herba; MMF, Massa Medicata Fermentata; PS, Phaseoli Semen; SC, Siberia Cocklebur; AH, Artemisiae Herba; AAS, Armeniacaeamarum Semen

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