

# BIOACTIVE COMPOUND PROFILING OF MULBERRY DRIED FRUIT POWDER AND MULBERRY FRUIT PULP POWDER OF MR2 VARIETY THROUGH GC-MS FOR FOOD PRODUCT DEVELOPMENT

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**Short running title:** Bioactive profiling of mulberry MR2 variety powders

## ABSTRACT:

Mulberry fruits are widely used as food source and its cultivated almost all over the world. Berries from mulberry known for their nutritional and medicinal value. In this research, GC-MS based bioactive compound profiling of MR2 variety fruit powder and pulp were investigated. The GC-MS based phytochemical characterization of MR2 (*Morus alba*) revealed vast number of bioactive compounds including, phenolics, fatty acids, terpenoids, alkaloids, carbohydrates derivatives and other compounds. In this study aims that, to utilized the underutilized fruits from farmer's field. Because, many farmers from South India usually cultivated MR2 in their field. MR2 is one of the high fruit yielding varieties in India. But farmers are left the fruit underutilized in the field as waste. The pulp (residue) of the fruit always discarded after extraction of juice, but it also contains large numbers of phytochemicals. This study shows the bioactive compounds and their therapeutic value (Antimicrobial, Antioxidant, Anti-inflammatory and Antibacterial activity) of mulberry fruits from MR2 variety for food product development and gain additional income to the farmers.

**KEYWORDS:** Mulberry fruits, MR2, GC-MS, Anti-inflammatory, Therapeutic value

## INTRODUCTION:

Sericulture is an important agro-based industry that involves the cultivation of mulberry plants and the rearing of silkworms for silk production. Among the various components of sericulture, mulberry (*Morus spp.*) occupies a central position as it serves as the sole food source for the domesticated silkworm, *Bombyx mori*. Traditionally, mulberry cultivation has been primarily focused on leaf production to support silkworm rearing (Gautam *et al.*, 2022). However, in recent years, increasing attention has been directed toward the utilization of other economically valuable parts of the mulberry plant, particularly its fruits, due to their exceptional nutritional and therapeutic properties. Mulberry plant is cultivated around the worldwide and its primarily belongs to the three major species named as *Morus alba* (White), *M. nigra* (black), *M. rubra* (red) (He *et al.*, 2018). Difference species and varieties of mulberry have notable variations in their nutritional profile and bioactive compound profiles

Moreover, fruits and vegetables especially deep coloured fruits are rich source for bioactive compounds, such as phenolic compound, anthocyanins, amino acids vitamins and minerals, which are best for human immune. These compounds help to prevent various chronic diseases including cancer, diabetes and cardiovascular diseases (Wallace *et al.*, 2020, Shabbir *et al.*, 2021, Dhalaria *et al.*, 2020). Mulberry fruits exhibit anti-microbial and anti-inflammatory, immune- supporting, blood sugar regulation and oxidative stress- reducing effects (Uyak *et al.*, 2024, Chen *et al.*, 2022).

MR2 is one of the most popular mulberry varieties grown in India, and it is known for its high yield, resistance to disease, and quality fruit. These fruits have good colour, good taste and a high content of bioactive compounds (Deepa *et al.*, 2022). But the fruits of MR2 were underutilized and wasted in farmer's field. The nutrient-rich fruits of mulberry have significant commercial potential and are extensively processed into various value-added products, including jams, jellies, pulp, sauces, cakes, wines, fruit powders, and natural food colorants, thereby enhancing their economic value (Singhal *et al.*, 2010, Maskan *et al.* 1998).

Gas chromatography-mass spectrometry (GC-MS) method used for profiling volatile and non-volatile compounds due to its high sensitivity, separation efficiency and compatibility with spectral libraries like NIST and Wiley (Rubaye *et al.*, 2017). It contains two different analytic techniques combined to single method of analysing the mixture of compounds. Components of mixture separate by Gas chromatography and analysis of each compound by mass spectrometry (Deng *et al.* 1964; Hedeji and Xu 1988; Shi *et al.* 2005). To maintain the efficiency in recovering wide range compounds, such as phenols, alkaloids, fatty acids, terpenoids and its polarity methanol were used as extraction solvent (Špánik, I., and Machyňáková, 2018). In this study, aims to profiling the bioactive compounds for food product development from mulberry fruit powder and mulberry fruit pulp powder.

## **MATERIAL AND METHOD:**

### **Fruit collection:**

The mulberry fruits at ripened stage were collected from MR2 plants at famers field at Annur area, Coimbatore in 11.23° N latitude and 77.11° E longitude. Collected fruits are stored in the airtight food grade containers to avoid spoilage and damage and transported from garden to lab (Figure 1 and 2).

### **Preparation of Sample:**

Mulberry fruit dried using fruit dryer for 2 days at 50°C and ground well for mulberry fruit powder (Figure 3). Extract the pulp from fruit juice by filtering with muslin cloth and kept in fruit dryer for 2 days at 50°C until it dries well and dried pulp were ground well for mulberry fruit pulp powder (Figure 4). Mean 70ml of juice filtered from 100 gm of mulberry fruit and mean 29 gm of pulp were extracted with five replications.

### **Extraction Method and GC-MS screening:**

Extraction of sample for GC-MS done by Soxhlet apparatus and 25 g of both fruit powders are extracted with 250 ml of analytical-grade methanol for 6 h at 60° (Figure 5). Rotary evaporator used for concentrate the filtrate from Soxhlet apparatus and stored in amber vials at 4° (Figure 6). Perkin elmer clarus SQ8C system equipped with a DB-5MS standard non-polar capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness) used for GC-MS analysis.

## **RESULTS:**

The bioactive compound in the mulberry dried fruit powder and dried pulp powder extracts was identified by gas chromatography mass Spectroscopy and it shows wide range of compounds such as, Phenolics, fatty acids, terpenoids. From all these, only metabolites with documented positive biological activity that's helps to human health management were selected for further detailed interpretation. Compounds shown in the tables are reported in the literature for their antimicrobial, antioxidant, anti-inflammatory properties.

### **Dried fruit powder of MR2:**

The GC-MS of dried fruit powder revealed vast number of bioactive compounds they are strongly associated with health benefits. The phenolic compound presented dried fruit powder is 1,2,3-Benzenetriol. Fatty acids were presented, notably 2-Hexyldecanoic acid, α-Oxo heptanoic acid, Pentanoic acid propyl ester, Nonanoic acid, Hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid methyl ester, α-Oxo heptanoic acid. Carbohydrates (D-Galactose & di-Glyceraldehyde dimer) are presented. Other compounds, indole alkaloid (Tryptamine and 1-Imidazole-2-methanol), Amino acid ester (D-Alanine, N-propargyloxycarbonyl-, isohexyl ester) 3-Hexanol, 5-Hydroxymethylfurfural, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one were presented. (Table 1) (Figure 7).

### **Dried fruit pulp powder of MR2:**

The GC-MS analysis was revealed different types of phytochemicals presented in MR2 mulberry fruit pulp powder. Butylated Hydroxytoluene and beta.-Tocopherol are the detected phenolic compounds presented in the pulp powder anti-inflammatory and neuroprotective activity. D-Limonene, β-Caryophyllene and alpha.-Terpineol are presented terpenes and terpenoids. Carbohydrates (d-Mannose) has presented. Fatty acids (Palmitic acid, Oleic Acid, 9,12-Octadecadienoic acid, Ricinoleic acid) also presented. Steroids and triterpenoids (Lup-20(29)-en-3-one, 5.alpha.-Cholest-8-en-3-one, 6.alpha.-hydroxy-14 methyl-, acetate) were presented. (Table 2) (Figure 8).

## **DISCUSSION:**

The GC-MS based bioactive compounds characterization of MR2 Variety (*Morus alba*) fruit powder and pulp powder revealed a diverse profile of phenolics, fatty acids, terpenoids, alkaloids derivatives, many of which are associated with therapeutic potential and biological activity. The phenolic compounds of both mulberry fruit powder and pulp powder such as 1,2,3-Benzenetriol, Butylated Hydroxytoluene and beta.-Tocopherol are previously documented as antimicrobial/anti-viral, antioxidant and anti-inflammatory activity (Kim *et al.*, 2026, Thbayh *et al.*, 2021, Jiang *et al.*, 2022). Fatty acids (2-Hexyldecanoic acid, α-Oxo heptanoic acid, Pentanoic acid propyl ester, Nonanoic acid, Hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid methyl ester, Palmitic acid, Oleic Acid, 9,12-Octadecadienoic acid, Ricinoleic acid) were documented as antioxidant, anti-inflammatory and antimicrobial effects (Obukhova *et al.*, 2024, Mazumder *et al.*, 2020, Krishnappa *et al.*, 2024). Carbohydrates (D-Galactose & di-Glyceraldehyde dimer, d-Mannose) documented anti-U TI efficiency, probiotic effect (Scribano *et al.*, 2020, Teshome *et al.*, 2022). Alkaloids (Tryptamine and 1-Imidazole-2-methanol) are documented as antimicrobial, antioxidant, anti-inflammatory, antifungal and antibacterial activities ( Zhang *et al.*,

2026, Agista *et al.*, 2022, Andrei *et al.*, 2021). Terpenoids (D-Limonene,  $\beta$ -Caryophyllene and  $\alpha$ -Terpineol), Steroids and triterpenoids (Lup-20(29)-en-3-one, 5. $\alpha$ -Cholest-8-en-3-one, 6. $\alpha$ -hydroxy-14 methyl-, acetate) are documented as antioxidant, anti-inflammatory, antimicrobial effects (Masyita *et al.*, 2022, Del Prado-Audelo *et al.*, 2021, Ikezu *et al.*, 2025). These compounds are not only contains above mentioned therapeutic value and biological activity, also contains various types of pharmaceutical and therapeutic potential.

## CONCLUSION:

In this reasearch, the bioactive compond profiling of MR2 shows the phytochemicals status of mulberry fruit powder and pulp powder, including terpenoids, pyrazines, fatty acid derivatives, alkaloids, phenolics with beneficial therapeutical value and bilogical activity. Especially, they shown anti-inflammatory, antimicrobial activity and other therapeutic potential based on the various documented findings. From these findings, MR2 fruit powder and pulp powder contains more therapeutic phytochemicals which is essential for human health and its leads to developing the food products to avoid the underutilized fruits from farmer's field. In addition, these food products gain additional income to farmers. Further studies using targeted analytical techniques and biological assays are required to confirm the identified compounds and validate their specific biological activities.

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## Author Contributions

**Pavithra N:** Conceptualization, Methodology, Analysis, Writing original draft.

**Priyadharshini P:** Methodology, Analysis, & Review

**Muruges K A:** Methodology, Writing – Editing.

**Arulmari R:** Data Collection & Analysis,

**Geetha P:** Supervision

## Conflict of Interest

The authors do not have any conflict of interest.

## Data Availability Statement

Data will be made available on request.

## Ethics Statement

The present study did not require ethical approval

## Informed Consent Statement

The sensory evaluation study was conducted with the approval of the individual.

## Clinical Trial Registration

This research does not involve any clinical trial

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**Figures:**

**Fig. 1: Collection of fruit from farmer's field**



**Fig. 2: MR2 variety fruits stored in food grade containers**



**Drying of fruits**



**Grinding**

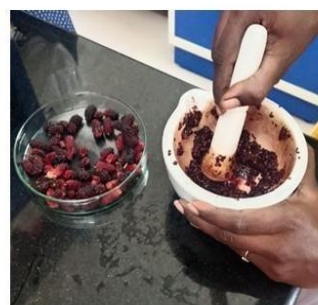


**Dried Fruit powder**

**Fig. 3: Preparation of dried fruit powder**



**Weighing of fruits**



**Grinding**



**Extraction**



**Dried pulp powder**



**Extracted Pulp**



**Fig 4: Preparation of dried fruit pulp powder**



**Fig. 5: Soxhlet extraction for GC-MS analysis**



**Fig. 6: Fruit extract from Soxhlet**

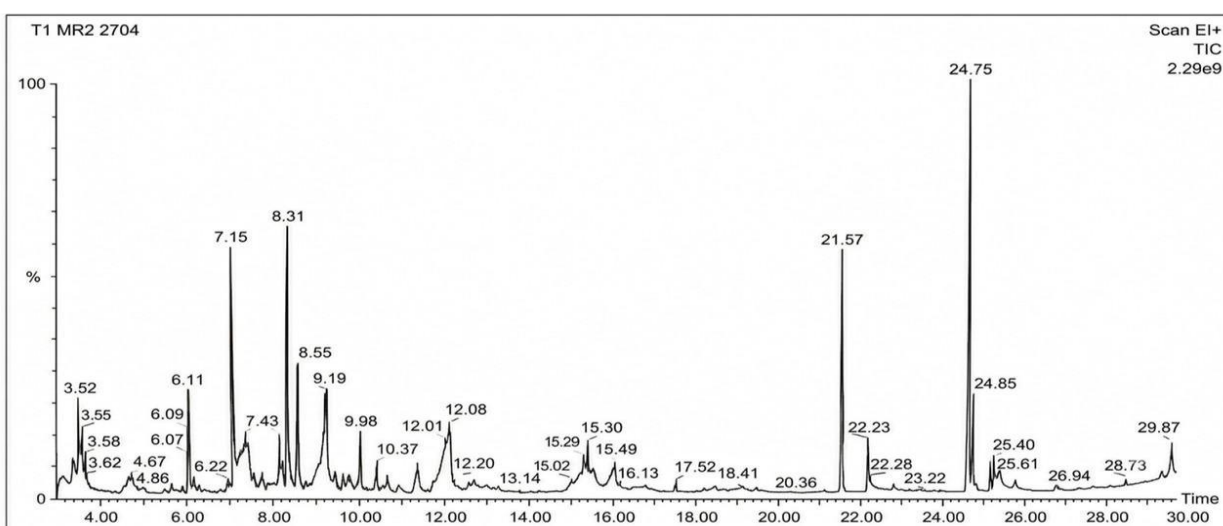


**Fig. 7: GC-MS Chromotogram of MR2 variety fruit dried powder**

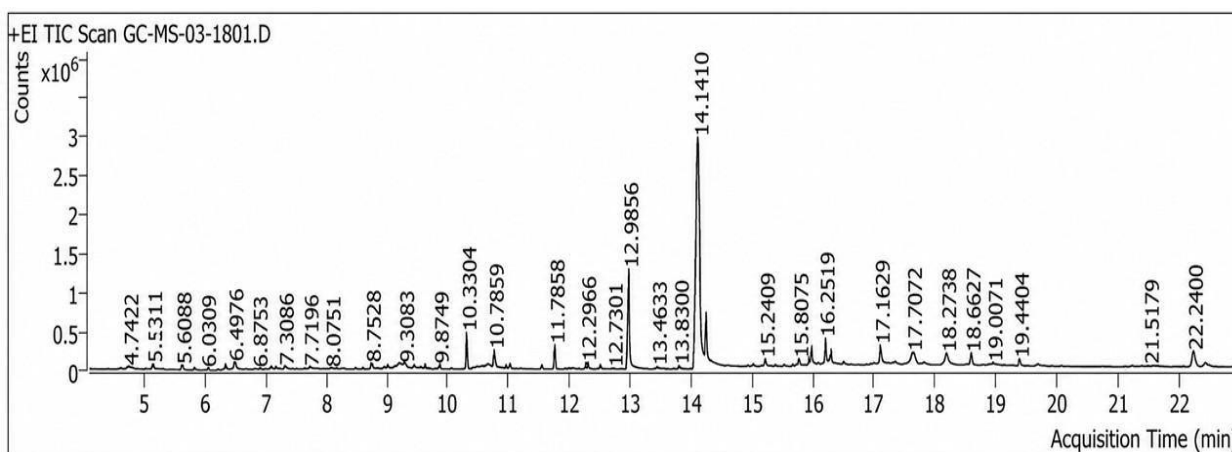


**Fig. 8: GC-MS Chromotogram of MR2 variety fruit pulp dried powder**

| S.No | Retention Time (min) | Compound Name                                      | Molecular Formula                               | Molecular Weight (g/mole) | Chemical class                                  | Biological Property                                   |
|------|----------------------|----------------------------------------------------|-------------------------------------------------|---------------------------|-------------------------------------------------|-------------------------------------------------------|
| 1    | 3.113                | 1H-Imidazole-2-methanol                            | C <sub>4</sub> H <sub>6</sub> N <sub>2</sub> O  | 98.10                     | Imidazole derivatives                           | Anti-inflammatory, Anti-depressant                    |
| 2    | 3.519                | di-Glyceraldehyde dimer                            | C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>   | 180.16                    | Dioxane derivatives                             | Sugar moiety and Preservative                         |
| 3    | 4.714                | D-Galactose                                        | C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>   | 180.15                    | Monosaccharides                                 | Anticoagulant, Antiviral, Antioxidant                 |
| 4    | 6.110                | D-Alanine, N-propargyloxycarbonyl-, isohexyl ester | C <sub>13</sub> H <sub>21</sub> NO <sub>4</sub> | 255.31                    | Amino acid derivatives                          | Antibacterial                                         |
| 5    | 7.145                | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one  | C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>    | 144.12                    | Pyranones / Dihydropyranones                    | Antioxidant                                           |
| 6    | 7.145                | Tryptamine                                         | C <sub>10</sub> H <sub>12</sub> N <sub>2</sub>  | 160.21                    | Indoleamines / Alkaloids                        | Antimicrobial, Antioxidant and Antibacterial activity |
| 7    | 8.146                | 3-Hexanol                                          | C <sub>6</sub> H <sub>14</sub> O                | 102.17                    | Branched-chain Fatty Acids                      | Antimicrobial and Antibacterial                       |
| 8    | 8.146                | 2-Hexyldecanoic acid                               | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>  | 112                       | Branched-chain Fatty Acids                      | Antimicrobial, Antibacterial, Antifungal              |
| 9    | 8.311                | 5-Hydroxymethylfurfural                            | C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>    | 126.11                    | Furanones / Furans                              | Antioxidant, Anti-proliferative activities            |
| 10   | 9.191                | α-Oxo heptanoic acid                               | C <sub>7</sub> H <sub>12</sub> O <sub>3</sub>   | 144                       | Alpha-Keto Acids                                | Antiviral                                             |
| 11   | 9.976                | Pentanoic acid, propyl ester                       | C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>   | 144.21                    | Aliphatic Esters                                | Antioxidant                                           |
| 12   | 10.617               | 1,2,3-Benzenetriol                                 | C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>    | 126.11                    | Polyphenols / Trihydroxybenzenes                | Antimicrobial, Antioxidant, Antinematicidal           |
| 13   | 12.697               | Nonanoic acid                                      | C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>   | 158.23                    | Medium-chain Fatty Acids                        | Antimicrobial, Antifungal                             |
| 14   | 21.571               | Hexadecanoic acid, methyl ester                    | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>  | 270.45                    | Fatty Acid Methyl Esters                        | Antioxidant, Antibacterial                            |
| 15   | 29.895               | 9,12-Octadecadienoic acid (Z, Z)-, methyl ester    | C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>  | 294.47                    | Polyunsaturated Fatty Acid Methyl Esters (PUFA) | Anti-inflammatory                                     |

**Table 1:** GC-MS profiling of MR2 variety fruit dried powder

| S.No | Retention Time (min) | Compound Name | Molecular Formula               | Molecular Weight (g/mole) | Chemical class | Biological Property                |
|------|----------------------|---------------|---------------------------------|---------------------------|----------------|------------------------------------|
| 1    | 5.1311               | D-Limonene    | C <sub>10</sub> H <sub>16</sub> | 136.24                    | Monoterpene    | Antioxidant, Anti-inflammatory and |

|    |         |                                                                   |                                                |        |                                    |                                                                        |
|----|---------|-------------------------------------------------------------------|------------------------------------------------|--------|------------------------------------|------------------------------------------------------------------------|
|    |         |                                                                   |                                                |        |                                    | Antimicrobial effect, Flavour compound                                 |
| 2  | 6.4976  | Benzyl acetate (Acetic acid, phenylmethyl ester)                  | C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>  | 150.17 | Aromatic ester                     | Antimicrobial and Antiolytic                                           |
| 3  | 6.8753  | .alpha.-Terpineol                                                 | C <sub>10</sub> H <sub>18</sub> O              | 154.25 | Monoterpene alcohol                | Anti-inflammatory, Antimicrobial and Anti-cancer, Natural preservative |
| 4  | 9.0306  | β-Caryophyllene                                                   | C <sub>15</sub> H <sub>24</sub>                | 204.35 | Sesquiterpene                      | Anti-inflammatory, Neuroprotective potential, Antiolytic               |
| 5  | 9.2083  | d-Mannose                                                         | C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>  | 180.16 | Monosaccharide                     | Anti-U TI efficiency and Probiotic effect                              |
| 6  | 9.6416  | Butylated Hydroxytoluene                                          | C <sub>15</sub> H <sub>24</sub> O              | 220.35 | Synthetic phenolic antioxidant     | Antimicrobial/Anti-viral and Antioxidant                               |
| 7  | 12.8412 | Oleic Acid                                                        | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub> | 282.46 | Monounsaturated fatty acid         | Cardioprotective, Anti-inflammatory                                    |
| 8  | 12.9856 | Palmitic acid (n-Hexadecanoic acid)                               | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> | 256.42 | Saturated fatty acid               | Anti-inflammatory, Antioxidant                                         |
| 9  | 14.1410 | 9,12-Octadecadienoic acid (Z,Z)-                                  | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub> | 280.45 | Omega-6 polyunsaturated fatty acid | Cholesterol regulation and Cell membrane integrity                     |
| 10 | 15.2409 | Ricinoleic acid                                                   | C <sub>18</sub> H <sub>34</sub> O <sub>3</sub> | 298.46 | Hydroxy Fatty Acid                 | Laxative Effect and Anti-inflammatory, Antimicrobial                   |
| 11 | 16.2519 | Abietic acid                                                      | C <sub>20</sub> H <sub>30</sub> O <sub>2</sub> | 302.45 | Diterpenoid                        | Antimicrobial, Antifungal and Anti-inflammatory                        |
| 12 | 17.1740 | 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester       | C <sub>21</sub> H <sub>38</sub> O <sub>4</sub> | 354.4  | Linoleic acid monoglyceride        | Antimicrobial, Antioxidant and Anti-inflammatory                       |
| 13 | 17.7072 | Lup-20(29)-en-3-one                                               | C <sub>30</sub> H <sub>48</sub> O              | 424.70 | Lupane triterpenoid                | Potent Anti-diabetic Activity, Anti-inflammatory, Anticancer           |
| 14 | 19.4404 | .beta.-Tocopherol                                                 | C <sub>28</sub> H <sub>48</sub> O <sub>2</sub> | 416.68 | Vitamin E tocopherol               | Cell membrane protection, Anti-inflammatory and Neuroprotective        |
| 15 | 22.2400 | 5.alpha.-Cholest-8-en-3-one, 6.alpha.-hydroxy-14 methyl-, acetate | C <sub>30</sub> H <sub>48</sub> O <sub>3</sub> | 456.71 | Steroid derivative                 | Anti-inflammatory, Anticancer and Anti microbial                       |

**Table 2:** GC-MS profiling of MR2 variety fruit pulp dried powder