Increasing Health Benefit of Wild Yam (*Dioscorea hispida*) Tuber by Red Mold (Angkak) Fermentation

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Abstract. Detoxification of cyanogen is very important in cyanide containing tubers such as wild yam (*Dioscorea hispida*). Principally, cyanogen detoxification is by converting cyanogenic glycoside into acetone cyanohydrin and further converted into free HCN that is easily removed by heating or soaking. Conversion of cyanogenic glycoside into free HCN is catalyzed by beta glucosidase enzyme (linamarase) in linamarin deglycosilation and alpha-hydroxynitril liase (HNL) in acetone cyanohydrin degradation into free HCN and acetone. Endogen linamarase is found in the tubers and exogenous linamarase might be from microbes. It is supposed that fermentation of wild yam by angkak will reduce cyanide level. Angkak or Red Mold Rice (RMR) is a product of rice fermentation using *Monascus sp* mainly *M. purpureus*. Beside red pigment, angkak fermentation also produces a variety of secondary metabolites such as lovastatin, mevinolin, and citrinin. Monacolin K (lovastatin) from *Monascus purpureus* is an inhibitor for HMG-CoA reductase in cholesterol biosynthesis. Monacolin K production is higher in *Dioscorea* substrate compared to rice. RMD (Red Mold Dioscorea) reveals higher anti-cholesterol activity and anti-hypertension than red mold rice. RMD also exhibits antioxidant, anti-diabetic, anti-obesity, and induces cancer cell apoptosis and does not reveal mutagenic and toxicity. In RMD, monascinin and ankaflavin have a role in reducing cholesterol. It is expected that wild yam fermentation by angkak will reduce cyanide level and produce health beneficial secondary metabolites.

Keywords: Angkak, Cyanide, Lovastatin, Red Mold *Dioscorea*, Wild Yam

1. Introduction
Wild yam (*Dioscorea hispida*) is a family of yam tubers or *Dioscorea* that contains bioactive compounds in the form of diosgenin of 96 ppm yam, 0.034% dioscorin, and water soluble polysaccharides (WSP) 3.02% [1]. Dioscorin is a tuber storage protein which has a function as an antioxidant, anti-inflammatory, anti-insect, and antipathogen [2]. Liu et al. [3] states that dicoscin shows antihypertensive activity in *vitro*. In addition, dioscorin shows ACE inhibitory activity in *vitro* which is important in inhibiting hypertension [4]. WSP has biological activity as an antioxidant to protect DNA [5], immunostimulant [6], and antihypertensive activity because it binds to dicoscorin [7].
**Dioscorea** also contains diosgenin as a bioactive compound [8] which plays a role in reducing plasma and liver triglycerides [9]. Diosgenin (3b, 25R-Spirost-5-en-3-ol), is a plant sterol with a cholesterol-like structure [10]. According to Son et al. [11], diosgenin (steroidal saponins) has been used industrially as a medicinal ingredient and has a hypocholesterolaemia effect because it suppresses cholesterol absorption and increases its secretion. Also inhibits the enzyme HMG Co A reductase in cholesterol synthesis [12].

One of the main problems is that the wild yam tuber contains HCN of 84.26 ppm [13] and 379 - 739 ppm [14], and the levels of the types of cyanogenic compounds in wild yam has not been studied. Cyanogenic compounds include cyanogenic glucosides, acetone cyanohydrin, and free HCN [15]. The safe level of cyanide consumption is 10 ppm [16]. Cyanide consumption of 50-100 ppm causes acute poisoning and is also lethal [17]. Cyanogenic residues cause neurological disorder or paralysis [18, 19].

Some efforts have been conducted to detoxify the wild yam tuber, such as by immersion and boiling [13], limited heating [20], boiling, roasting, and soaking in running water [21]. Another method to detoxify is through fermentation as in cassava. Decreasing cyanide in cassava occurs during spontaneous fermentation in the production of agelima, gari, atieke, lafun, bikedi, fufu, pupuru in Africa and wikau maombo in Indonesia [22, 23, 24, 25, 26, 27]. It is expected that fermentation of wild yam will also reduce cyanide. Dioscorea can be fermented using red mold rice (Monascus purpureus) to produce Red Mold Dioscorea (RMD) [28, 29, 30]. RMD shows the ability as a cholesterol-lowering and antiarterosclerotic agent [28,29] and lowering blood pressure [30]. This is due to higher monacolin K production compared to rice substrate [28] and RMD contains higher γ-aminobutyric acid (GABA) and contains yellow pigments (monascin and ankaflavin) which are anti-inflammatory [31]. Therefore, to improve the functional properties of yam flour on health, yam tubers needs to be fermented using red mold rice. Angkak pigment is a mixture of pigments consisting of at least 6 pigments grouped by color. Red pigment consists of rubropunctamine and monascorubramine and is the most pigment; orange pigments are rubropunctatin and monascorubrin; and yellow pigments are monascin and ankaflavin [32]. The composition of the pigment depends on the strain of Monascus sp and the media used.

This paper is aimed to review the bioactive compounds of wild yam and their physiological effects on health benefits, the method of detoxification, and the possibility to increase health benefits of wild yam by rice mold fermentation.

### 2. Bioactive Compounds of Wild Yam

#### 2.1. Dioscorin

The tubers of the Dioscorea (yam) family have long been used in traditional Chinese medicine. The most water soluble protein in yam, which is dioscorin and its hydrolysate by using proteases reveal various biological activities. Dioscorin has the ability as an antioxidant even orally consumed, and approved as an anti-aging [33]. The stability of dioscorin has been approved by Liu and Lin [34] and shows that boiling and frying causes dioscorin to be denatured and difficult to dissolve. It means dioscorin changes during processing and this changes might affect its activity. Dioscorin is also reported to have immunomodulatory activity [3], antioxidants [35], improves metabolic metabolism in obese rats and decreases systolic blood pressure [36]. The results of hydrolysis of dioscorin with pepsin, also showed an ability to reduce blood pressure [37]. This means that the fermentation of yam with red mold rice might hydrolyze dioscorin and increases its activity. At present dioscorin has been studied in nanoparticle size that are easily absorbed in digestive tract [38].

Structurally, dioscorin from Dioscorea alata (greater yam) consists of dioscorin A (BM 33 kDa) and dioscorin B (BM 31 kDa) with the main structure of α helices for dioscorin A and antiparallel β sheets for dioscorin B. Dioscorin is known to have amino acid sequences N-terminal VEDFYSIEGNPNGPENWGN. In the yam protein there is an oligomeric structure that is possibly related to the disulfide bond (32 kDa) [39]. Hou et al. [40] mentioned the molecular weight of dioscorin from D. batatas is 28 kDa. Chen and Lin [41] reported BM 31 kDa for various cultivars.
2.2. Diosgenin
Diosgenin is an aglycone from steroidal saponins, dioscin, in yam. Diosgenin is the result of hydrolysis of saponins. Dioscin levels in yams can reach 2.7% while diosgenin is around 0.004% in yams that are cultivated, and 0.12-0.48% in wild yams. Diosgenin has long been used as a raw material for steroid medicines. Diosgenin is reported to have a hypocholesterolaemia effect by suppressing cholesterol absorption and increasing its secretion [11]. Diosgenin also has the ability to induce apoptosis of cancer cells [42], inhibits skin aging [43], is antithrombotic, lowers blood sugar, enhances the immune system and is antioxidant [44]. Yeast fermentation with red mold rice may increase diosgenin due to hydrolysis of dioscin during fermentation.

2.3. Water Soluble Polysaccharides or Mucilage
Water soluble polysaccharides (WSP) or water soluble fiber or mucilage from yam ia a complex between manan and protein [45] which contains glycoprotein and food fiber [46]. Mucilage from yam shows antioxidant activity [47, 48], inhibits the activity of angiotensin converting enzyme (ACE) [49], antimicrobial [46], and activity hypoglycemic [50] and immunomodulators [45]. The study of Zhang et al. [51] showed that the result of mucilage degradation of D. opposita increased its activity as an antioxidant and antimutagen because of lower molecular weight and more uronic acid. It is strongly supposed that fermentation of yam with red more rice might lead to hydrolysis of mucilage and increases its activity.

2.4. Cyanogenic Compounds
Cyanogenic compounds in tubers are found in three forms which are cyanogenic glycosides, hydroxynitrile or acetone cyanohydrin, and free HCN [52]. Cyanogenic glycosides and endogenous enzymes are located in different locations (Figure 1). Linamarin and lotaustralin are present in cell vacuoles and endogenous enzymes (β-glucosidase or linamarase and liase) present in cell walls [53]. The characteristics of linamarin, acetone cyanohydrin, and free HCN from cassava are shown in Table 1.

Table 1. Characteristics of linamarin (cyanogenic glucoside), acetone cyanohydrin, and free HCN

| Characteristics            | Linamarin | Acetone Cyanohydrin | Free HCN |
|----------------------------|-----------|---------------------|----------|
| Optimum pH                 | 5.5 – 7.3 | > 5                 | No data  |
| Optimum temperature (°C)   | 55        | > 35                | No data  |
| Solubility in water        | Soluble   | Soluble             | Soluble  |
| Boiling point (°C)         | No data   | 82                  | 25.6     |
| [55, 56, 57, 58, 59]        |           |                     |          |

The damage to the cell wall causes contact between linamarin and the linamarase enzyme and converts cyanogenic glucoside into glucose and cyanohydrin aglycone [57]. Cyanohydrin or hydroxynitrile acetone is relatively stable under acidic conditions, but spontaneously decomposes.
rapidly at pH> 5.0 or catalyzed by hydroxynitrile liase (HNL) which is found specifically in the leaves [56]. The boiling point of HCN is 26 °C causing HCN to evaporate easily in the drying process [60]. The changing of linamarin into free HCN is shown in Figure 2.

![Figure 2. The changing of linamarin to free HCN [61]](image)

### 2.5. Detoxification of Cyanogenic Compounds

The cyanogen detoxification process is very important in the processing of cyanide containing tubers such as wild yam. In principle, detoxification is changing of cyanogenic glycosides into acetone cyanohydrin which is then converted to free HCN, and then is removed by evaporation, heating, or dissolution. The process of converting cyanogenic glycosides to free HCN is catalyzed by the enzyme beta glucosidase (linamarase) in the process of deglycosylation of linamarin and the enzyme α-hydroxynitrile liase (HNL) in the process of acetone cyanohydrin degradation to free HCN and acetone, or spontaneously degraded (McMahon et al., 1995) [53]. Endogenous linamarase is found in tubers while exogenous linamarase can be derived from microbes. Microbes that are often associated with linamarase production during cassava fermentation are lactic acid bacteria (BAL) and yeast [62, 63].

Ferraro et al. [64] reviewed that the method includes grating, grinding (breaking down of tissue) is a very efficient method in removing cyanide due to cell breakdown. It leads linamarin in vacuoles to contact with beta glucosidase (linamarase) in the cell wall. Therefore, there is the conversion of cyanogenic glucoside into acetone cyanohydrin and then to free HCN that is easily removed by dissolving in water or evaporation. Drying and fermentation after drying (heap fermentation) is less efficient because the tubers are sliced so that some cells remain intact [65]. Heap fermentation helps break down cyanogenic glycosides by microbes during fermentation [66, 67]. Boiling is inefficient because linamarase is inactive, but boiling is far more efficient (% decrease in cyanide) than roasting, steaming, or frying (15-20% decrease in cyanogen). Pressing after fermentation or grating is efficient (70-95% decrease in cyanide) because free cyanide or residues can be removed [68].

Angkak (*Monascus purpureus*) is a fungi that also produces beta glucosidase enzyme in submerged fermentation with maximum activity at pH 5.5 and temperature 50°C [69]. This enzyme is produced extracellularly whose production is induced and controlled by the presence of carbon (glucose) [70]. It is strongly supposed that fermentation of yam with red mold rice can reduce cyanogen, one of which is caused by the extracellular beta-glucosidase enzyme produced by *M. purpureus*.

### 2.6. Rice Mold Fermentation and Health Benefits

Angkak or Red Mold Rice (RMR) is a rice fermentation product using *Monascus* sp, especially *M. purpureus*. Angkak is originated from China and is widely used as a food colouring. In addition to red pigment, red mold rice fermentation produces various secondary metabolites such as lovastatin, mevinolin, citrinin, and vitamins [71, 72]. Angkak pigments also have antioxidant activity which activity depends on the intensity of the pigment colour [73]. Monacolin K (lovastatin) from Monascus purpureus is an inhibitor for HMG-CoA reductase in biosynthesis of cholesterol. Lovastatin also shows inhibition of cancer cells [74], potential use for autoimmune therapy [75], protecting endothelial cells [76] form oxidized LDL [77], improve osteoporosis [78], and prevent inflammation [79]. Angkak pigments also show antioxidant activity [80].
Besides lovastatin, Monascus purpureus also produces pigments including two yellow pigments, monascin and ankaflavin; two orange pigments, rubropunctatin and monascorubrin; and two red pigments, rubropunctamine and monascorubramine. Solid and submerged fermentation is a fermentation process for the production of Monascus pigments. The advantage of solid fermentation is that it can be directly used as a colouring agent. Factors that influence red mold rice fermentation are the source of carbon, nitrogen, pH, temperature, minerals, the presence of oxygen, and the presence of other microorganisms [72].

Fermentation medium is a critical factor in the growth of M. purpureus which also influences pigment production. Metal ions such as Zn$^{2+}$ and Mg$^{2+}$, and a number of amino acids (glycine, leucine, tryptophan) might improve the carbon source transfer process. Zn is a cofactor for enzymes in carbohydrate and nitrogen metabolism by Monascus sp [72]. Factors that must be controlled in red mold rice fermentation is the presence of citrinin. Citrinin is a mycotoxin polyketide produced by Monascus sp and is usually a contaminant for RMR [81]. Substrate with too high or low water content is not suitable for Monascus growth [28, 82].

2.7. Rice Mold Dioscorea

Monacolin K production is higher in Dioscorea substrate compared to rice [28]. RMD (Red Mold Dioscorea) shows higher anticholesterol activity compared to rice substrate [29], higher antihypertensive activity compared to RMR [31], has antioxidant and antidiabetic ability (Shi et al, 2012) [83], induces apoptosis of oral cancer cells [84, 85], and antiobesity [86], and does not show mutagenic and toxic properties [87]. In RMD, monascin and ankaflavin play a role in reducing cholesterol [88].

3. Conclusions

Wild yam contains some bioactive compounds such as dioscorin, diosgenin, and water soluble polysaccharides. However, this tuber also contains cyanogenic compounds as a toxicant. Detoxification of wild yam involves the changing of cyanogenic glycosides into acetone cyanohydrin which is then converted to free HCN. Free HCN is easily removed by evaporation, heating, or dissolution. However, the main problem is the conversion from cyanogenic glucoside into acetone cyanohydrin and then into free HCN. Fermentation is supposed to decrease cyanogenic compounds of wild yam. Fermentation of wild yam with red mold rice is expected to increase the activity of its bioactive compounds and also decrease cyanides. It is supposed that health beneficial effects of wild yam also increase by red mold fermentation.

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