Development of a User-Friendly Curcumin (*Curcuma Longa)* and Piperine (*Piper Nigrum*) Incorporated Capsule as a Food Supplement

Hettiarachchige A. Prasad[1*]; Thushari Liyanage[2]; S.B. Navaratne[1]

[1]Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka

[2]Central Research Station, Department of Export Agriculture, Matale, Sri Lanka

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ABSTRACT

Black pepper (*Piper nigrum* L.) has been used by humans for thousands of years. People in ancient Sri Lanka used black pepper for various purposes, including traditional medicine and culinary uses. A spice in the Piperaceae family known as black pepper (Piper nigrum L.) contains an alkaloid named piperine, which imparts the pungency of black pepper. Turmeric (*Curcuma longa* L.) has been used for culinary, cosmetic, and medicinal purposes. Curcumin (curcumin I) is the most essential principal bioactive compound present in turmeric naturally. Curcumin has been confirmed to have beneficial antioxidant, anti-inflammatory, antiviral, and antifungal actions. The combination of piperine and curcumin enhances the bioavailability and absorption of curcumin. The grounded black pepper and turmeric powder samples were used to extract oleoresins of black pepper and turmeric. Techniques were adapted to crystallize and purify piperine and curcumin from turmeric and pepper. A lipid-based formulation combining piperine and curcumin was developed, as well as a soft gelatin-based encapsulation method. According to proximate analysis, the average moisture content of ground black pepper samples taken from the Matale area was 13.6392 ± 0.3643 %, and the average moisture content of turmeric powder samples was 13.9877 ± 0.2651 %. The average yield of piperine was 8.3115 ± 3.0359 %, and the average yield of crude curcuminoids was 11.1020 ± 0.9580 %. Piperine and curcumin were loaded into fish oil and cocoa butter using ethanol as a carrier. Lipid-based piperine and curcumin-loaded drug formulations were carried out using soft gelatin encapsulation.

Key words: Piperine, Curcumin, Oleoresins, Soxhlet extraction, Crystallization

INTRODUCTION

Pepper is also known as the "king of spices" and originated in South India. Due to its prized trade, pepper is also referred to as "black gold." Vietnam, Malaysia, India, Indonesia, and Brazil are the primary producers of pepper. According to genetic variability, *Piper nigrum* L. and wild relatives can be found in Sri Lanka. Turmeric has been cultivated intentionally due to its vivid yellow color, which was initially used as a dye in cosmetics and for beautification. Later, it was used for culinary and medicinal purposes. It became an Indian-originated spice in China, as well as in East and West Africa.

Piperine is the main bioactive compound found in black pepper, and it has been confirmed that piperine has specific pungent properties and many biological effects. Piperine enhances general human health by enhancing antioxidant, anti-metastatic, immunomodulatory, hepatoprotective, and antitumor activities. Additionally, it has been confirmed that piperine can improve the bioavailability of various drugs. According to these therapeutic potentials, it is vital to incorporate piperine into health-enhancing medicinal formulations (Stojanovi, 2019). Curcumin (curcumin I) is considered the principal curcuminoid of turmeric, and other curcuminoids are also referred to as curcumin II (desmethoxy curcumin) and curcumin III (bis-desmethoxy curcumin). Curcuminoids are bright yellow and are used as a food colouring agent. As a food additive, it is called E 100 under food colours. Curcumin is not a toxic compound for humans; it has beneficial antioxidant, anti-inflammatory, antifungal, and antiviral properties. The action of molecules caused by inflammation can be inhibited by curcumin(Akram et al.,

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2010). It has been confirmed that a combination of piperine with curcumin is vital to enhance the absorption and bioavailability of curcumin.

MATERIAL AND METHOD

According to the method described by Tiwari and colleagues, Soxhlet extraction was used to obtain oleoresin from black pepper (Tiwari et al., 2020). About 10 g of ground black pepper (*Piper nigrum* L.) and 200 ml of ethanol were added to a round-bottom flask. After that, the Soxhlet extraction was carried out for 15 h at 80 ºC. Then, the extract was heated for one hour to concentrate, and a membrane filtration was carried out for 4-5 h with rinsing with hot (60-70 ºC) ethanol. Then, approximately 20 mL of 10% KOH solution was mixed with the hot filtrate, and a second membrane filtration was performed on the mixture.The filtrate was kept for 24 hours in an ice bath and then for four hours in a hot air oven to obtain piperine crystals. According to the methodology described by Naresh D. Joshi and colleagues (2021), approximately 10 g of turmeric powder (Curcuma longa L.) was measured, and the Soxhlet extraction apparatus was used to obtain the oleoresin of turmeric by adding 200 mL of ethanol as the solvent. Soxhlet extraction was carried out for 15 h at 80 ºC, and the extract was concentrated by mild heating for one hour. Then, the extract was kept for 24 hours to remove excess ethanol, and the crude curcuminoids were collected. Crude curcuminoids were added to hexane at a concentration of 1.00 g of crude extract per 25 mL of hexane (Rajapakse, 2021). After that, the mixture was stirred gently for 20 minutes and then left for 12 hours. Then, the solution was stirred at 600 rpm for three hours to obtain a dissolved curcumin solution. After that, the solution was centrifuged for 15 minutes, and the supernatant (hexane) was removed at 40 °C for 2 hours. The sediment (Curcumin) was then collected.

Taguchi's orthogonal array prepares the lipid-based formulations with three selected variables: curcumin, piperine, and medium. The acceptable daily intake (ADI) of Curcumin is 0-3 mg/kg body weight per day, according to the European Food Safety Authority (EFSA) (*Www.Bfr.Bund.De*, 2021) (Rajapakse, 2021). The ADI of piperine is 15-20 mg/day, and these requirements are considered when determining the available amount of these two bioactive compounds and the dosage of the prototype capsule (Wadhwa et al., 2014). Samples were prepared as shown in Table 1 below.

Table 1: Three-factor sample preparation table

Experiment no.	Curcumin (mg)	Piperin (mg)	Medium
1	Low (0.0000)	Low (0.1500)	Low (Fish oil)
2	Low (0.0000)	High (0.2000)	High (Cocoa butter)
3	High (0.0300)	Low (0.1500)	High (Cocoa butter)
4	High (0.0300)	High (0.2000)	Low (Fish oil)
5 (Control)	-	-	Fish oil
6(Control)	-	-	Cocoa butter

According to Table 1, the amount of curcumin and piperine was precisely measured and dissolved in 15 mL of ethanol. After that, the medium, as indicated in Table 2.1, was added to this solution and maintained at 40 °C for four hours to remove excess ethanol until the weight remained constant. Final formulations were manually injected into soft gelatin capsules and then heat-sealed.

RESULTS AND DISCUSSION

Moisture content

The ground black pepper and turmeric powder were taken and subjected to determine the average moisture content. The average moisture contents are presented in Table 2.

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Table 2: Average moisture contents of black pepper and turmeric from Sri Lanka

Sample	Average moisture content %
Black pepper	13.6392 ± 0.3643
Turmeric	13.9877 ± 0.2651

According to Table 2, the average moisture content of ground black pepper samples is 13.6392 ± 0.3643 %, and the average moisture content of turmeric powder samples is 13.9877 ± 0.2651 %, whereas resulted in values resulted from the method as Dean and Stark Toluene Distillation Method (AOAC 17th edition 2000 Official Method 986.21). The recommended maximum moisture content of ground black pepper is 12 % (w/w), and the value of turmeric powder is 10 % (w/w)(*European Spice Association Quality Minima Document*, n.d.). Therefore, there is a notable difference between reference values and analyzed moisture. Spices such as black pepper and turmeric are hygroscopic, allowing atmospheric moisture to interact with them. A higher moisture content may lead to fungal growth, and mycotoxins may be present in the final dietary supplement (Pasanen et al., 1991). Therefore, high moisture content can affect the quality of the ultimate product of nutritional supplements.

Crystallization and purification

The crude ethanol extract of oleoresin black pepper consists of piperine and isomers of acidic and resinous substances. Adding a 10% KOH solution is essential to prevent the co-precipitation of piperine and the precipitation of resins and chavicine. Thus, particles such as brownish-yellow solid gummy materials with cloudiness were observed as the precipitation of these insoluble residues. Resins and chavicine, except piperine, are retained through membrane filtration.

Fig 1: crystalline piperine

The crude curcuminoids contain curcumin I (curcumin), curcumin II, and curcumin III. The colour of curcuminoids and curcumin may change with different pH and environmental conditions, such as ambient light. Monika and colleagues (2018) have suggested that non-composite resins are incorporated with crude curcuminoids to create a reddish colour(Access, 2018). Anting Wulandari and colleagues (2020) stated that the colour changes of curcumin occur under pH 2-13, and at alkaline conditions, the colour changes to a brownish orange. However, the colour of curcumin is relatively stable in acidic and neutral conditions. Accordingly, according to N. Petchana and colleagues (2020), yellow curcumin can be obtained at pH levels of 5-7; at pH levels of 8-11, the color may change to reddish-brown. The stability of curcumin decreases as the pH value increases, resulting in the formation of an amber-colored ferulic acid and a whitish-yellow vanillin. Also, curcumin is photodegradable in an aqueous medium, and in dark conditions, it is also self-degradable.

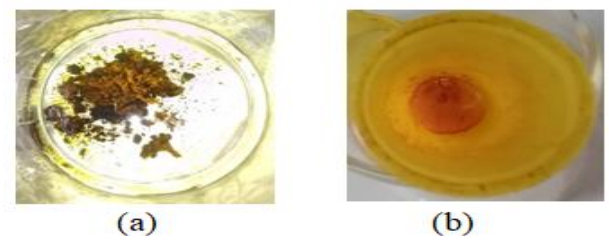

Fig 2: (a) crude curcuminoids, (b) crystalline curcumin

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As a food-grade solvent, n-hexane was added with the crude curcuminoids to dissolve curcumin, except for other resinous parts in the n-hexane. Resinous components are generally insoluble in hexane and under neutral pH (pH = 7.0) conditions of n-hexane; the colour of the crude was observed to be brownish orange, as shown in Figure 2(a). The most non-polar one is curcumin I, compared to curcumin II and curcumin III (Jafarinejad-farsangi et al., n.d.). Due to the insoluble nature of non-composite resins, the solubility of curcumin in n-hexane is very high, and a solution of curcumin was obtained in a cloudy yellowish orange suspension under the corresponding pH condition of n-hexane, concentration of particles, and purity. Figure 2(b) shows the crystalline curcumin gained with the centrifugation of the curcumin-dissolved n-hexane.

Average yield of piperine and curcuminoids(crude)

Average percentages of yields were calculated for piperine and crude curcumin, and the total weight of curcumin was measured on a dry basis. The summarized results data are presented in Table 3 below.

Table 3: Average yield of piperine and crude curcuminoids

Crystalline compound	Average yield %
Piperine	8.3115 ± 3.0359
Curcuminoids (crude)	11.1020 ± 0.9580

According to Anshul Tiwari and colleagues (2020), the piperine yield % of black pepper is 3.2%. ER, Jansz, and colleagues stated that the yield of piperine is 2-7% in black pepper in Sri Lanka (Section, 1983). Inadequate KOH may cause the precipitate of chavicine and resinous substance with piperine after the membrane. According to John Zachariah and colleagues (2015), the yield of curcuminoids ranges from 2.5% to 6.0% (Leela, 2015). The reference values show a significant difference from the analysed yield of crude curcuminoids. A sticky nature of crude curcuminoids was observed, and the remaining solvent bound with debris may cause an increase in the yield of oil curcuminoids.

Lipid-based formulation of curcumin and piperine

Piperine and curcumin-loaded fish oil formulations were filled into soft gelatin capsules. A prototype capsule developed with a fish oil-based formulation is shown in Figure 3 below.

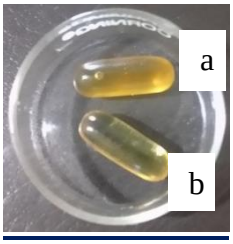

Fig 3: Soft gelatin capsules (a) piperine and curcumin-loaded capsules, (b) commercially available fish oil capsule

After 14 days, a slightly shrunken appearance was observed. Autoxidation can happen in lipid-based formulations due to entrapped air bubbles. Additionally, the soft gelatin capsule's transparent cover may allow curcumin oxidation through photo-oxidation.

CONCLUSION

Crystalline, needle-like structures can be observed in a yellowish color through the crystallization and purification of piperine. Reddish-brown crude curcuminoids, resulting from oleoresin turmeric, and an orange-colored, powder-like precipitate, similar to curcumin I, were obtained through purification. Piperine and curcumin can be loaded into lipolytic solutions with ethanol, which will be removed as a secondary process. Fish

oil and cocoa butter can be used to develop lipophilic solutions incorporating piperine and curcumin. Soft gelatin encapsulation is suitable for lipid-based formulations of piperine and curcumin.

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