

Fortification of Dark Chocolate with Encapsulated Phytochemicals from Black Cardamom and Black Cumin and its Quality Evaluation

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Abstract

The spices and herbs are well known for its flavor and health benefits. The use of Phytochemicals from the Black cardamom (*Amomum sublatum*) and Black cumin (*Nigella sativa*) enriches the novel food products. Encapsulation is a widely used technique in food industries to entrap the volatile and bioactive components into a wall material and is used to enhance food products. This study aims to Formulate Dark chocolate with the incorporation of Encapsulated Phytochemicals and the Nutritional, and functional properties of the product will be assessed. Encapsulated phytochemicals from these spices were then incorporated to dark chocolate, a food product known for its health benefits. Combining dark chocolate's natural phytochemicals with the therapeutic properties of the encapsulated phytochemicals will result in a fortified product with enhanced antioxidant properties. The findings of this study show that the formulated dark chocolate contain favorable nutritional parameters, including energy content (812.5 ± 3.21 kcal), protein (7.04 ± 0.06 g), fat (48.5 ± 0.3 g), calcium (7.33 ± 0.11 %), carbohydrates (86.45 ± 0.49 g), moisture (2.58 ± 0.22 %), ash (3.90 ± 0.07 %), and notably high levels of total phenol content (4596.46 ± 3.49 mg/ml) and antioxidant activity. It can be summarized from the current investigation that the formulated Dark chocolate incorporated with Encapsulated Phytochemicals from Black cardamom and Black cumin can be used by all age groups people to improve the antioxidant scavenging activity to prevent chronic diseases caused by free radical formation.

Key words: Dark chocolate, Encapsulation, Phytochemicals, Black cardamom, Black cumin

Spices and herbs are used mainly in the world cuisines. Spices play a major role in providing consumers with aroma, flavor, taste, and various health benefits. The spices contain phytochemicals, and essential oils that have unique characteristics to prevent cancer, Alzheimer and other metabolic syndromes. Spices are a potent source of antioxidants, which prevent the oxidative stress caused due to the generation of excessive free radicals in the body. The spices contain certain bioactive compounds which can lead to compromise the quality of the product. These bioactive compounds in spices will lead to degradation due to oxygen, moisture, light, etc., which can be prevented by Encapsulation, and drying techniques [18].

The *Amomum sublatum* is known as Black Cardamom. It has a sweet aroma and bioactive compounds that cure various illnesses such as cardiovascular, diabetes, oxidative stress, and inflammation. The Phytochemicals such as flavonoids, and polyphenols are highly present in the Black cardamom. Cardamom contains potent antioxidants, a lot of research proposed that dietary antioxidants have been more effective compounds than pure compounds in preventing oxidative stress-related pathologies [38]. Black cardamom and Black cumin are cultivated in high amounts in West Bengal, and Gujrat [4], [16].

The Black cumin also called *Nigella sativa* is famous for its culinary uses and is historically precious in traditional medicines. Black cumin has various traditional uses and medicinal properties including antioxidant, anti-inflammatory, immunomodulatory, anticancer, antimicrobial, cardioprotective and hepatoprotective properties. Black cumin seed essential oils contain thymoquinone, thymol, and nigellidine which are responsible for the pharmacological effects and therapeutic benefits. The extracts of black cumin have shown to stimulate phagocytic activities thereby boosting the immune system [16].

The Encapsulation technique is widely used in novel food product development [2]. The bioactive phytochemicals are encapsulated and entrapped inside a wall material thereby protecting the Encapsulated Phytochemicals from environmental conditions which will lead to oxidation of the bioactive compounds. The Encapsulated Phytochemicals can be used to fortify various food products to enhance their antioxidant capacity.

Dark chocolate has various health benefits. The dark chocolate is naturally rich in flavonoids and these will protect from certain heart disease. Different types of chocolate contain different amounts of theobromine and dark chocolate contains higher levels of theobromine. Certain research suggests that

dark chocolate will reduce the risk of heart disease. The compounds in dark chocolate will exhibit different therapeutic activities and have benefits including alleviation of hypertension, antioxidant properties, etc. [22].

This study aims to Fortify Dark chocolate with Encapsulated Phytochemicals from Black cardamom and Black cumin. Dark chocolate has various health benefits and is naturally rich in flavonoids that prevent certain heart diseases. Dark chocolate can be used as an ideal platform to incorporate Encapsulated Phytochemicals. The Fortified dark chocolate with Encapsulated Phytochemicals was further evaluated for its Nutritional and functional properties.

The main objective of the study is to:

1. To extract and encapsulate the Phytochemicals from Black cardamom and Black cumin.
2. To Fortify the Dark chocolate incorporated with Encapsulated Phytochemicals.
3. To analyze the Nutritional composition and Functional properties.

MATERIALS AND METHODS

Selection and procurement of ingredients

The ingredients used in this study are locally available and provide potential health benefits. The ingredients such as Black cardamom (*Amomum sublatum*) Black cumin (*Nigella sativa*) cocoa powder, icing sugar, and Butter are procured from the local supermarket.

Ethical consideration

This study entitled “Fortification of Dark Chocolate Incorporated with Encapsulated Phytochemicals Black Cardamom and Black Cumin: Analysis of Nutritional & Functional Properties” has been approved by the Institutional Human Ethics Committee (IHEC) with the protocol no SDNBVC/IHEC/2023/11- conducted on 20.11.2023 by the Department of Home Science- SDNB Vaishnav College for Women, Chromepet, Chennai-44.

Extraction and encapsulation of phytochemicals from black cardamom and black cumin

The Phytochemical compounds from the Black cardamom and Black cumin were extracted employing Soxhlet extraction technique suggested by [30] and Encapsulated using the Spray drying method suggested by [3]. The maltodextrin was used as a wall material to entrap the bioactive compounds. Figure 1 depicts the steps involved in entrapment of phytochemicals.

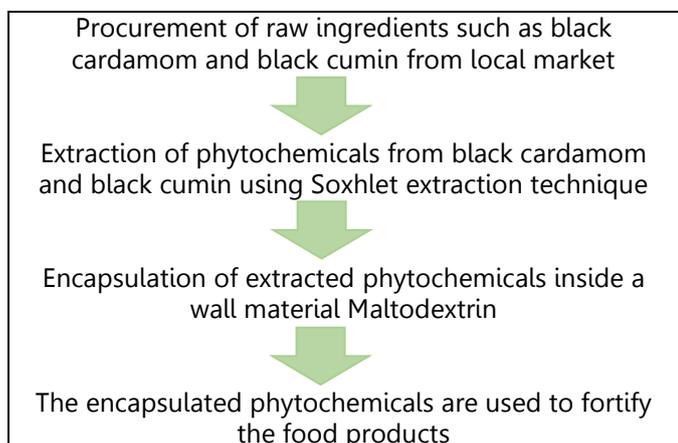


Fig 1 Extraction and encapsulation of phytochemicals from black cardamom and black cumin



Fig 2 Encapsulated phytochemicals from black cardamom and black cumin using spray drying

Fortification of dark chocolate with encapsulated phytochemicals from black cardamom and black cumin

Dark chocolates are well known for their various health benefits. Dark chocolate with a high content of cocoa beans has the highest concentration of polyphenols and flavonoids compared to other food sources. Therefore, dark chocolate can be considered a functional food that can deliver health benefits with exceptional taste [7]. Considering the nutritional profile, dark chocolate was selected by the researcher to incorporate encapsulated phytochemicals. The formulation of Dark chocolate with the incorporation of Encapsulated Phytochemicals was adopted from the methods suggested by [35] as depicted in (Fig 2) and three treatments (T1, T2, T3) were tried and subjected to sensory evaluation and found to have appreciable sensory quality. Therefore, further T1 was subjected to further quality evaluation. The ingredients used in T1 are depicted in (Table 1).

Table 1 Ingredients used in the formulation of dark chocolate incorporated with encapsulated phytochemicals from black cardamom and black cumin

Ingredients	Treatment
Cocoa powder	50g
Icing sugar	30g
Butter	45g
Encapsulated phytochemicals from black cardamom and black cumin	5g
Total	130

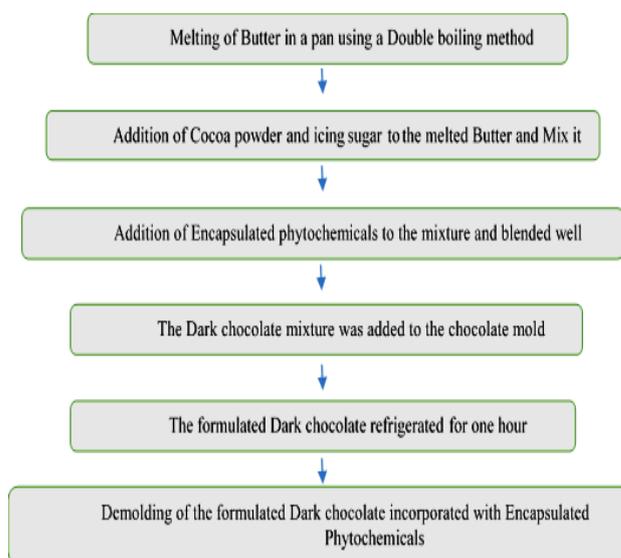


Fig 3 Steps involved in the formulation of dark chocolate incorporated with encapsulated phytochemicals from black cardamom and black cumin



Fig 4 Dark chocolate incorporated with encapsulated phytochemicals

RESULTS AND DISCUSSION

Nutritional analysis of the fortified dark chocolate incorporated with encapsulated phytochemicals

The fortified Dark chocolate incorporated with Encapsulated Phytochemicals was assessed for nutritional composition such as Energy, Protein, Fat, Fatty acid profile, Carbohydrate, Moisture, Ash, and Minerals such as Calcium, Iron, Phosphorous, and Magnesium. The results obtained are represented in (Table 4) and explained below with a relevant literature review.

Table 4 Nutrient composition of the fortified dark chocolate incorporated with encapsulated phytochemicals

Nutrients	Treatment
Energy (Kcal)	812.5±3.21
Protein (g)	7.04 ± 0.06
Carbohydrate (g)	86.45 ± 0.49
Fat (g)	48.5 ± 0.3
Calcium (mg)	7.33 ± 0.11
Moisture (%)	2.58 ± 0.22
Ash (%)	3.90 ± 0.07
Calcium	8.33 ± 0.11 %
Iron	11.1 ± 0.1 mg
Phosphorous	297.7 ± 0.6mg
Magnesium	245.31 ± 0.01mg

All values are the mean of triplicate determination ± standard deviation
 NS- non-significant, S* - Significant at 5% level, and
 S** - Significance at 1% level

Energy

The present study investigated the Nutritional composition of the Fortified Dark chocolate incorporated with Encapsulated Phytochemicals. The energy content observed in the formulated dark chocolate was found to be 812.5 ± 3.21 Kcal per 100gm. The energy value of formulated Dark chocolate with encapsulated Phytochemical was high when compared to the values obtained in the previous study by [27] who formulated Dark chocolate with pepui mesocarp (519.71 kcal/100g). The difference in the calorific value could be due to the variation of ingredients used in both the studies.

Protein

The protein content of the food is the necessary macronutrient for the body's various processes and operations. The protein content of the sample was found to be 7.04 + 0.06g per 100g. A study by [25] obtained 6.37 ± 0.01g of protein in the GABA enriched Dark chocolate and a study by [19] who formulated Chocolate incorporated with Encapsulated probiotic strains reported similar protein values (6.81g). Furthermore, in Dark chocolate fortified with wheat germ by [1], the protein content of the fortified product was found to be 7.5g which showed a similar occurrence of protein found in Formulated Dark chocolate incorporated with Encapsulated Phytochemicals from black cardamom and black cumin.

Carbohydrate

Carbohydrates contribute to the energy source for human beings. The carbohydrate content of formulated dark chocolate was found to be 86.45 ± 0.49g. The high carbohydrate content could be due to various factors, including the effect on starches or sugars present in the sample. The result of the study was compared with a previous study conducted by [26]. In that study, cereal bars coated with chocolate, which included encapsulated *Lactobacillus acidophilus*, had a carbohydrate value of 51.09±0.03g. The study by [12] who formulated Dark

Nutritional analysis of the fortified dark chocolate incorporated with encapsulated phytochemicals

The fortified Dark chocolate was evaluated for its Nutritional properties such as Energy, Protein, Fat, Fatty acid profile, Carbohydrate, Moisture, Ash, Calcium, Iron, Phosphorous, and Magnesium were analyzed and the methods suggested by [37] and exhibited in (Table 2).

Table 2 Proximate composition analysis technique and references

Parameters	Test methods
Energy	FAO method
Protein	AOAC 2000
Fat	AOAC gravimetric method
Fatty acid profile	Bartkieniè <i>et al.</i> (2021d) [5]
Carbohydrate	FAO method
Moisture	AOAC, 2000
Ash	AOAC, 2000
Calcium	IS 1656 : 2007
Iron	IS 1656 : 2007
Phosphorous	IS 1656 : 2007
Magnesium	IS 1656 : 2007

Functional property analysis of the fortified dark chocolate incorporated with encapsulated phytochemicals

The functional properties such as Phytochemical analysis (Flavonoids, Alkaloids, Saponins, Tannins, Phenols, Terpenoids, Steroids, Glycosides), Total Phenolic content, and Antioxidant activity of the product were assessed. The method followed for the same is depicted in (Table 3).

Table 3 Functional properties of fortified dark chocolate incorporated with encapsulated phytochemicals

Functional properties	Method
Phytochemical analysis	Shaikh, <i>et al.</i> (2020) [31]
Total Phenol content	Folic-Ciocalteu Assay
Antioxidant property	DPPH radical scavenging activity [23]

Statistical analysis

The data obtained from the Nutritional properties such as Energy, Protein, Fat, Fatty acid profile, carbohydrate, Ash, Moisture, and Mineral composition, and Functional properties such as Phytochemical analysis, Total Phenol content, and Antioxidant properties of the formulated Dark chocolate incorporated with Encapsulated Phytochemicals from Black cardamom and Black cumin were subjected to statistical analysis mean, standard deviation. In which the value of individual items in the series is obtained from the arithmetic average. The data was coded and determined.

chocolate with different cocoa varieties reported a carbohydrate content of $53.36 \pm 0.7g$. However, this value was lower than the value obtained from the dark chocolate incorporated with encapsulated phytochemicals in the current study. This can be due to variations in the ingredients used in the current investigation.

Fat

The fats are essential for various biological processes. The fat content in the food product will be estimated using a soxhlet apparatus. The fat content of the fortified dark chocolate was found to be $48.5 \pm 0.3g$ which was found to be higher when compared with Milk chocolate incorporated with Encapsulated *Spirulina sp* $18.27 \pm 1.60g$ as reported by [13]. The another study by [19] showed that the fat content of dark chocolate was $34.28g$ and a study by [34] who formulated dark chocolate with pomegranate peel documented the fat content of the product as $70.5g$. The variations in the fat content in the previous research study could be due to the variation in the butter content as an ingredient in dark chocolate preparation.

Fatty acid profile

The fatty acid profile was analyzed for the formulated Dark Chocolate. The fatty acid profile includes Saturated fatty acids, MUFA, PUFA, Trans fatty acids, and total unsaturated fatty acids. The results obtained from the current investigation are depicted in the (Table 5). The results shows that the formulated Dark chocolate incorporated with Encapsulated Phytochemicals from Black cardamom and Black cumin was found to have a good amount of beneficial unsaturated fatty acid. The total unsaturated fatty acid was found to be $24.58 \pm 0.08\%$ and the MUFA and PUFA content was found to be 14.88 ± 0.07 , and 9.67 ± 0.06 respectively. The results obtained were compared with the study conducted by [5] in the Dark chocolate the MUFA 33.00% , PUFA 7.53% , and SFA 59.465% , and by the study [35] the MUFA 32.27% , PUFA 2.57% , and SFA 66.03% respectively. It can be concluded from the results obtained in the current study that the formulated product has a good amount of heart healthy fatty acid profile and also has a lower amount of SFA%.

Table 5 The fatty acid profile of the formulated dark chocolate incorporated with encapsulated phytochemicals

Fatty acid profile	Values
Saturated fatty acid	12.22 ± 0.17
MUFA	14.88 ± 0.07
PUFA	9.67 ± 0.06
Total unsaturated fatty acid	24.58 ± 0.08

Moisture and ash

Substantial moisture content was observed in the formulated dark chocolate ($2.58 + 0.22\%$) when compared with previous research findings. The results obtained were compared with the study by [1] which contains 6.2% of moisture content. This decrease in moisture content could be indicative of appropriate processing methods employed during the formulation of the product. The formulated dark chocolate incorporated with encapsulated Phytochemical was found to have an ash content of $3.90 \pm 0.07\%$. A similar result of Ash content (2.12%) was found in the study who formulated probiotic chocolate formulation by [24] and by [25] in the GABA-enriched Dark chocolate formulation ($2.31 \pm 0.14\%$).

Mineral composition of the fortified dark chocolate

The Mineral composition of the formulated Dark chocolate was assessed and the results are depicted in (Table 4).

Calcium is an essential mineral required by the human body. The formulated product exhibited notable calcium content ($7.33 \pm 0.11\%$). The calcium content obtained was compared with the study by [17] obtained $1443\text{ mg}/100g$ of calcium in probiotic chocolate which was found to be higher than the formulated Dark chocolate. The variation in the results could be due to the difference in the proportion of milk solids utilized for the preparation of the chocolates.

Iron has a various function in the body; it serves as a carrier of oxygen to the tissues. The Iron content of the Formulated Dark chocolate incorporated with encapsulated phytochemicals was $11.1 \pm 0.1\text{mg}/100g$. A similar result was found in the study by [11] who documented similar the iron content in dark chocolate ($11.24 \pm 0.25\text{mg}$). The magnesium content of the formulated Dark chocolate was found to be $245.31 \pm 0.01\text{mg}/100g$ respectively. The Phosphorous content of the formulated dark chocolate was found to be $297.7 \pm 0.60\text{ mg}/100g$ respectively. A similar accordance was found in the study by [11] $297.63 \pm 9.37\text{mg}$ and it was merely similar to the formulated dark chocolate. From this, it can be concluded that the formulated Dark chocolate has many essential mineral contents and was found to be Nutritious. Overall, the results highlight the efficacy of the treatment in modulating the nutritional profile of the samples, with significant improvements observed in energy, protein, carbohydrate, calcium, and ash content, along with a reduction in moisture.

Functional properties of fortified dark chocolate incorporated with encapsulated phytochemicals

The Functional Properties such as qualitative analysis of phytochemical, Total phenol content, and antioxidant properties of the formulated Dark chocolate incorporated with encapsulated phytochemicals were analyzed and results are exhibited in (Table 6). Dark chocolate formulated without incorporating encapsulated phytochemicals is treated as a control sample to compare and conclude the effect of incorporation on the functional properties of the final product.

Table 6- Results of qualitative phytochemical analysis of dark chocolate incorporated with encapsulated phytochemicals

Phytochemical	Control	Treatment
Flavonoid	-	-
Alkaloid	-	-
Saponin	-	-
Tannin	+	++
Phenol	+	++
Terpenoid	+	++
Steroid	-	-
Glycoside	-	-

+ - Low presence, ++ - Moderate presence, +++ - High presence

a. Phytochemical analysis

In general, plant extract naturally contains certain bioactive compounds, which are referred to as phytochemicals and nutraceuticals have received incredible attention among health-conscious consumers [36]. The results of qualitative analysis of phytochemicals revealed the presence of Tannins, Phenols, and Terpenoids in the fortified Dark chocolate. The Phytochemicals have significant antioxidant potential due to their beneficial effect on human health [14].

b. Total phenol content

Total Phenols are naturally occurring compounds present in plants and have the potential effects to improve human health

by preventing cellular damage by inhibiting and delaying oxidative processes [9] and are used to treat or prevent various diseases. The total Phenol content of the formulated Dark chocolate incorporated with encapsulated Phytochemical was found to be $4596.46 \pm 3.49 \mu\text{g/ml}$ and it was compared with the control group which was found to be $2366.06 \pm 17.84 \mu\text{g/ml}$. These results show that the Phytochemical (phenol) content of the treatment group was found to be higher when compared to the control group. The results showed a statistically significant difference ($p < 0.01$) between the control and treatment groups. The most common phenolic compounds present in black cumin are p-hydroxybenzoic acid, protocatechuic acid, and chlorogenic acid [20] whereas black cardamom possesses phenolic compounds such as Protocatechuic acid, Gentisic acid, Caffeic acid, and Syringic acid as reported by [33]. A similar result was obtained in the study by [21] who formulated dark chocolate. A study by [28] documented the very high total phenolic content in the commercially available dark chocolate which was found to be $703.13 \text{ mg}/100\text{g}$. The variation in the

results could be attributed to the high total phenolic compounds that are naturally present in cocoa beans as reported by [6].

c. Antioxidant property

The Antioxidants are described as substances that specifically prevent or delay the oxidation of physiologically important molecules that quench the free radicals formed in the body [32]. Natural antioxidants present in spices have attracted the attention of researchers for many decades because of the rarity of their side effects and low toxicities [29]. The antioxidant activity of the treatment and control group was found to be $134.27 \mu\text{g/ml}$ and $122.6 \mu\text{g/ml}$ respectively. The superior antioxidant activity of the treatment group denotes a good radical scavenging ability of the formulated Dark chocolate incorporated with Encapsulated Phytochemicals from black cardamom and black cumin. The results summarize that formulated dark chocolate with encapsulated phytochemicals possessed strong antioxidant properties which is the added advantage of consuming dark chocolate regularly.

Table 7 Total phenol content

Control ($\mu\text{G}/\text{Ml}$)	Treatment ($\mu\text{G}/\text{Ml}$)	P- Value	Significance
2366.06 ± 17.84	4596.46 ± 3.49	0.00	S**

Table 8 Antioxidant properties of dark chocolate incorporated with encapsulated phytochemicals from black cardamom and black cumin

Samples	IC ₅₀ Value
Standard	99.05
Control	122.6
Treatment	134.27

CONCLUSION

The spices are widely used in many world cuisines. It contains Phytochemicals, and essential oils that have preventive characteristics to cancer and other metabolic syndromes. The Black cardamom and Black cumin were found to have higher Phytochemicals as the most bioactive components. The Phytochemicals are the most bioactive compounds that play a major role in preventing certain chronic diseases. Encapsulation is of growing importance in food industries to enhance the production of nutritious food products. The Phytochemicals present in the spices are more susceptible to oxidation or degradation, to prevent their bioactive compounds Encapsulation technique has been used. The inclusion of encapsulated phytochemicals extracted from black cardamom and black cumin into dark chocolate is a significant advancement in functional food development. This enriched dark chocolate is a highly nutritious option, carefully formulated to contain an enhanced profile of phytochemicals such as terpenoids, phenols, and tannins. These compounds

give the product potent antioxidant properties, making it a functional food capable of combating oxidative stress and reducing the risk of chronic diseases. The findings of this study underline the potential of fortified dark chocolate as a versatile dietary intervention accessible to individuals of all ages. By harnessing the preventive characteristics of phytochemicals against cancer, metabolic syndromes, and other chronic ailments, this innovative product offers a convenient and tasty way to improve overall health and well-being. Moreover, the successful application of encapsulation techniques in preserving the bioactive compounds within the spices highlights the importance of technological innovation in enhancing the nutritional quality of food products. As consumer interest in health-conscious choices continues to grow, fortified dark chocolate serves as a compelling example of how traditional ingredients can be used to create functional foods that promote optimal health and longevity. In conclusion, the fortified dark chocolate enriched with encapsulated phytochemicals from black cardamom and black cumin is set to make a meaningful impact on dietary habits and health outcomes. It offers a delicious and accessible way towards a healthier lifestyle for people of all ages.

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