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Potential Anti-Bacterial Activity of Defatted Coconut Meal Extracts Against Some Food Pathogenic and Food Spoilage Bacterial Strains

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THE ETHANOLIC and methanolic extracts of defatted coconut (*Cocos nucifera*) meal (after extraction, solvents used for extraction were eliminated using rotary evaporator, but extracts still named ethanolic and methanolic extracts along this research paper) were examined for their antimicrobial activity against some food borne pathogens (*Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* subsp. *aureus* and *Bacillus cereus*) and food spoilage microorganisms (*Bacillus cereus* and *Bacillus stearothermophilus*). The ethanolic extract showed higher phenolic contents (19.62 µg/g meal) than methanolic extract (11.84 µg/g meal). The ethanolic extract of defatted coconut meal gave a pronounced inhibition effect as well as bactericidal activity than the methanolic extract. This is most probably the reason of better antibacterial effect found by ethanolic extract. It was found that ethanolic extract of coconut meal has higher inhibition effect on *Staphylococcus aureus* subsp. *aureus, Salmonella typhimurium Bacillus cereus* and *Bacillus stearothermophilus*. However, methanolic extract showed higher inhibition activity *Salmonella typhimurium* > *Staphylococcus aureus*> *Bacillus cereus* >*Bacillus stearothermophilus*, respectively. It was found that ethanolic extract has bactericidal effect on all tested strains except *Escherichia coli*, which shows only inhibition effect. Transmission Electron Microscop (TEM) showed that, the ultrastructure was strongly altered in the form of aggregation of cell contents, cell wall disruption, transparent regions or high dense regions. It was concluded that, the combined effect of both bactericidal activity of the coconut meal and thermal treatments is promising as natural preservative agent of food staffs from spoilage by different bacterial strains.

Keywords: Coconut meal, extraction, antioxidant, phenolics, antibacterial.

Introduction

One of the palm species with major economic significance is the coconut, *Cocos nucifera*, which is grown primarily for its endosperm. Coconut by-products are produced as a result of the strong demand of virgin coconut oil in the worldwide market (Trinidad *et al*. 2006). Industrial by-products must be transformed into useful components for food compositions. The coconut meal produced when coconuts are processed either dry for oil extraction or wet for milk extraction. highlighted coconut meal as a valuable source of dietary fiber and revenue for the industrial sector. Because coconut meal is gluten-free, people with celiac disease can use it (Trinidad *et al*. 2006). High moisture and nutrient content as well as a pH close to neutrality result in the rapid microbial spoilage many kinds of food staffs (Chen *et al*. 2024). In the global market, there is a greater understanding of the connection between food intake and choice as well as a healthy lifestyle. Due to consumer preference for natural antioxidants over synthetic ones, food makers are currently attempting to incorporate

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natural sources. Previous study reported that, phenolic-rich foods, such as coconut (*Cocos nucifera*) fruit, showed potent antioxidant impact on health status (Adekola *et al*. 2017, Akhter *et al*. 2009, Appaiah *et al*. 2016 and Arivalagan *et al*. 2018). Natural antibacterial agent is preferred than synthetic due to low side effects. Coconut testa is a rich source of various polyphenolic and non-phenolic natural antioxidants, anti-inflammatory and antimicrobial compounds (Ojha *et al*. 2019). Antimicrobial activity was observed by both phenolic and non-phenolic compounds extracted from coconut testa. The rational of this study was to identify the phenolic compounds in the defatted coconut meal and evaluated its antioxidant activity, bacteriostatic or the bactericidal effect against some food borne pathogens and spoilage microorganisms. In addition, the impact of defatted coconut meal on the ultrastructure of the bacterial cells of the tested strains.

Materials and Methods

Preparation of defatted coconut meal

Defatted coconut meal was prepared by extracting oil from ground coconut meat with n-hexane using Soxhlet apparatus. The defatted meal was dried at room temperature and grounded and sieved to 60 mesh particles.

Proximate composition of defatted coconut meal.

The moisture content was determined using the air oven method, whereas the protein content was assessed using the Kjeldahl method. To estimate the crude protein percentage, the total nitrogen content was multiplied by a factor of 6.25, following the method described by Akasha *et al*. (2016). The ash content analysis involved incinerating approximately 2 g of coconut meal in a furnace set at 550°C for 8 h, in accordance with the protocol outlined by Rambabu *et al*. (2020).

The carbohydrate content was calculated using the following formula:

100 - (% moisture + % ash + % protein + % oil) (Nehdi *et al*. 2010).

Preparation of ethanolic and methanolic extracts of defatted coconut meal

The ethanolic and methanolic extraction were done by mixing defatted coconut meal (2 g) with ethanol or methanol at different concentration (20, 50, 80%) at room temperature. The extracts were then subjected to evaporation under vacuum at room temperature (extracts after preparation still named ethanolic or methanolic extracts along this research paper), then sterilized by filtration (Sartorious membrane filters, 0.2 µm, Germany). The sterilized extracts were diluted by double distilled water for the disc assay at 0.2% concentration. The growth curve was done in previous medium where extracts were placed.

Total phenolic content

The total polyphenolic content (TPC) of defatted coconut meal was determined by using Folin-Ciocalteu method Pérez *et al*. (2023).

Identification of phenolic compounds

The phenolic content of the sample was analyzed and identified using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) with an Exion LC™ AC system (USA) for separation and SCIEX Triple Quad 5500+ MS/MS system (Singapore) equipped with an electrospray ionization (ESI) for detection (Pietrzak, 2017).

Antioxidant activity of defatted coconut meal extract

Antioxidant activity of defatted coconut meal extract was determined using 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH). The extract was serially diluted (10–100 mg/ml) according to method of (Ahmad *et al*., 2021)

Antibacterial activity of defatted coconut meal extract

Different bacterial strains including *Escherichia coli* (DSMZ 5212), *Salmonella typhimurium* (DSMZ 5569), *Staphylococcus aureus* subsp. *aureus* (DSMZ 20231), *Bacillus cereus* (DSMZ 2302) and *Bacillus stearothermophilus* (DSMZ 297) were obtained from the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. These strains were subcultured on nutrient agar and stored at 4°C. All cultivations of the strains of *E. coli*, *Salmonella typhimurium*, *B. cereus* and *Staph. aureus* subsp. *aureus* were incubated at 37°C for 24 h, and at 55°C for 48 h for *B. stearothermophilus*.

Agar diffusion method (Davidson and Parish, 1989).

It has often been referred as the disc assay. The extracts were applied to agar plate, using an impregnated filter paper discs. The paper discs were sterilized separately and impregnated under sterile condition in tested extracts.

Assay of survival rate

According to (Schoenknecht *et al*., 1995),

inoculation of one of the tested bacteria into the liquid medium, containing the defatted coconut meal extract, followed by incubation all tested strains at 37°C except *B. stearothermophilus* at 55°C for 48 h followed by periodic sampling to determine growth or survival curve.

Transmission electron microscopy (TEM)

The changes in bacterial structure after treatment with defatted coconut meal extract were examined by TEM. The tested strain cells before and after the use of tested extracts were fixed with 3% glutaraldehyde in 50 mM phosphate buffer, pH 7.0, for 90 min at 4°C. The fixed cells were dehydrated through a concentration series of acetone and embedded in spurr medium (Spurr, 1969). After polymerization ultrathin sections were cut. The ultrathin sections were post-stained with 3% uranyl acetate and lead citrate (Venable and Coggeshall, 1965) and examined with a Philips EM 301 electron microscope (Philips, Eindhoven, The Netherlands) at 80 kv. Micrographs were taken at calibrated magnifications.

Results and Discussion

Proximate composition of defatted coconut meal

The chemical composition of defatted coconut meal revealed that, 6.55% moister, 20.9% protein, 7.0% oil, 3.2% ash and 62.35 carbohydrate.

Total phenolic content of defatted coconut meal

Total phenolic content was expressed as mg Gallic acid equivalent. By using different solvent gradients (20%,50% and 80%). It was found that, data presented in Fig.1 showed maximum extraction using 50% solvent as reflected by the highest phenolic content recorded of 19.62 µg/g meal using ethanol extraction, followed by 11.84 µg/g by the use of methanolic extraction. However, 20% and 80% showed approximately same yield extraction.

Identification of phenolic compounds of defatted coconut meal by LC/MS/MS

The relative retention times (RT) and concentrations of separated active compounds of defatted coconut meal were presented in (fig 2, table 1). It was found that, defatted coconut meal rich with active compounds**.** Naringenin showed highest level, followed by Ferulic acid, 3.4-Dihydroxybenzoic, Caffeic acid and Chlorogenic acid with concentrations of 6.965, 0.649, 0.585, 0.555 and 0.417 µg/g defatted coconut meal, respectively. However, other active ingredients as Gallic acid, rutin, Cuomaric acid, Hesperdine, reseveratrol are present with lower levels.

Antioxidant activity

The antioxidant activity of defatted coconut meal was determined by inhibition of DPPH as showed in (fig 3), which referred that, using 50% ethanol or methanol yielded higher antioxidant activity of 59,3% and 52.4% ethanolic and methanolic, respectively followed by 80% ethanol or methanol showed 53.9 and 50.6 and finally using 20% ethanol or methanol showed 48.4,42.6, respectively.

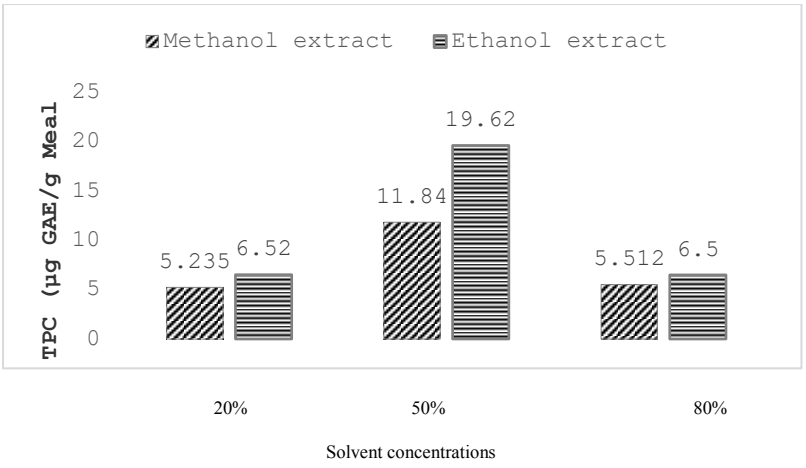

Fig. 1. Total phenolic content of defatted coconut meal using different concentration of ethanol or methanol solvent

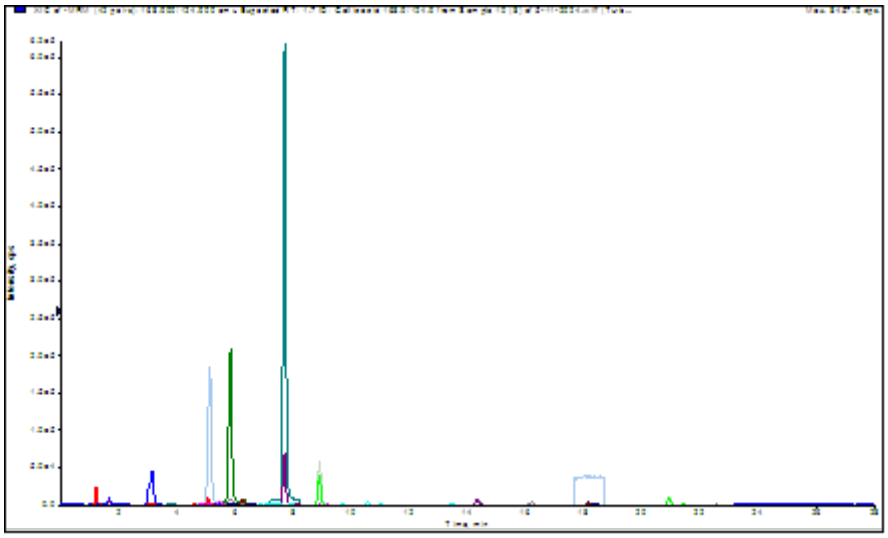

Fig. 2. LC/MS/MS chart showing the identified compounds of the defatted coconut meal.

TABLE 1. Identification active compounds of defatted coconut meal using LC/MS/MS.

Component Name	Expected RT	Area	RT	Calculated Concentration	Conc. (ug/g)
Gallic acid 168.9/124.9	1.67	4.59E+04	1.66	0.021	0.2126
Caffeic acid 178/135	5.83	1.50E+06	5.82	0.055	0.5544
Rutin 609/299.9	9.13	1.02E+04	9.12	0.017	0.1743
Coumaric acid 162.9/119	7.7	3.93E+06	7.69	0.034	0.341
Naringenin 271/151	20.98	9.11E+04	20.91	0.697	6.965
Querectin 301/151	18.16	N/A	N/A	N/A	ND
Ellagic acid 301/145	8.97	N/A	N/A	N/A	ND
3.4-Dihydroxybenzoic acid 152.9/109	3.13	4.88E+05	3.11	0.059	0.585
Hesperetin 301/136	22.62	N/A	N/A	N/A	ND
Cinnamic acid 146.9/77	18.16	N/A	N/A	N/A	ND
Methyl gallate 183/124	5.04	N/A	N/A	N/A	ND
Kaempferol 284.7/93	22.08	N/A	N/A	N/A	ND
Ferulic acid 192.8/133.9	8.89	4.15E+05	8.89	0.065	0.6485
Syringic acid 196.8/181.9	6.23	N/A	N/A	N/A	ND
Apigenin 269/151	21.47	N/A	N/A	N/A	ND
Catechin 288.8/109	5.05	5.89E+04	5.06	0.028	0.2827
Daidzein 253/132	16.22	3.41E+04	16.18	< 0	ND
Chlorogenic acid 353/191	5.1	1.41E+06	5.11	0.042	0.4169
Resveratrol 227/185	14.27	7.86E+04	14.31	0.019	0.1869
Rosmarinic acid 359.1/161	13.42	N/A	N/A	N/A	ND

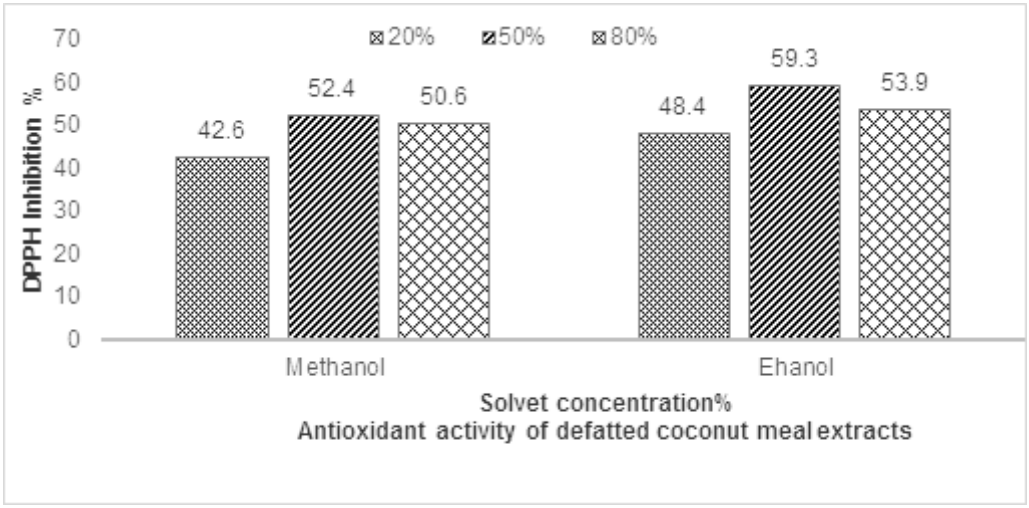

Fig. 3. Antioxidant activity using of defatted coconut extracts prepared with different concentrations of ethanol and methanol.

Antibacterial activity of the defatted coconut extracts

Table (2) shows the inhibitory or bactericidal effect of the defatted coconut meal extracts using the disc assay. Different concentrations of extracts were used (0.1%, 0.2% and 0.4%). The results of 0.2% concentration were showed in table (2). It was found that, ethanolic extract exerted inhibition effective when compared with the methanolic extraction. Ethanolic extract had a wider spectrum of inhibition on *Staphylococcus aureus* subsp. *aureus*, *Salmonella typhimurium, Bacillus cereus* and *Bacillus stearothermophilus* than methanolic extract. The *E.coli* showed moderate inhibition by both extracts. The methanolic extract caused maximum inhibition of *Salmonella typhimurium* and moderate inhibition of *Staphylococcus aureus* subsp. *aureus* and *Bacillus stearothermophilus*, this can lead to bactericidal effect but after longer time than ethanolic extract. This is similar to results of Ojha *et al*. (2019), who found that phenolic and non-phenolic compounds extracted from coconut testa showed different antimicrobial activities against different strains.

The inhibition or growth curve as described by (Schoenknecht *et al*., 1995) was to confirm and determine the exact effect of them on the tested microbial strains. Fig (4) showed the effect of 50% ethanolic and methanolic on *Escherichia coli* after incubation period of 24 h at 37°C. It is clear that both of them had inhibition effect. Fig. (5) shows the effect of 50% ethanolic and methanolic extract on *Staphylococcus aureus* subsp. *aureus*. It can be seen that both of them have bactericidal effect.

The bactericidal effect of ethanolic extract is effective than that of methanolic extract. They reach their bactericidal effect after 8 h and 18h incubation, respectively with the same previous strain. Fig. (6) Illustrates the effect of 50% ethanolic and methanolic extract on *Salmonella typhimurium*. They have both bactericidal effect and reach their bactericidal effect at the same time of incubation (8 h). The effect of 50% ethanolic and methanolic extract on *Bacillus cereus* was presented in Fig. (7). Only ethanolic extract kill *Bacillus. cereus* completely after 32 h of incubation. Methanolic extract has slight

TABLE 2. Antibacterial activity of ethanolic and methanolic defatted coconut extracts (0.2%) against some food borne pathogens using disc assay.

Extracts	Microorganisms				
	E.coli	*Staph. aureus subsp. aureus*	*Salmonella typhimurium*	*B. cereus*	*B.stearo-thermo-philus*
Ethanolic extract (50%)	++	+++	+++	+++	+++
Methanolic extract (50%)	++	++±	+++	+	++±

(+) Slight inhibition., (++) Moderate inhibition, (+++) Maximum inhibition.

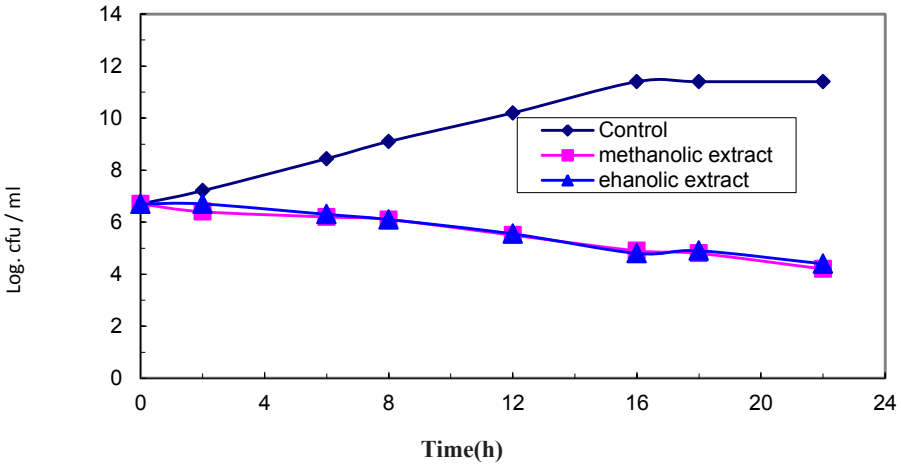

Fig. 4. Effect of 50% ethanolic and methanolic of defatted coconut meal extracts on Escherichia coli.

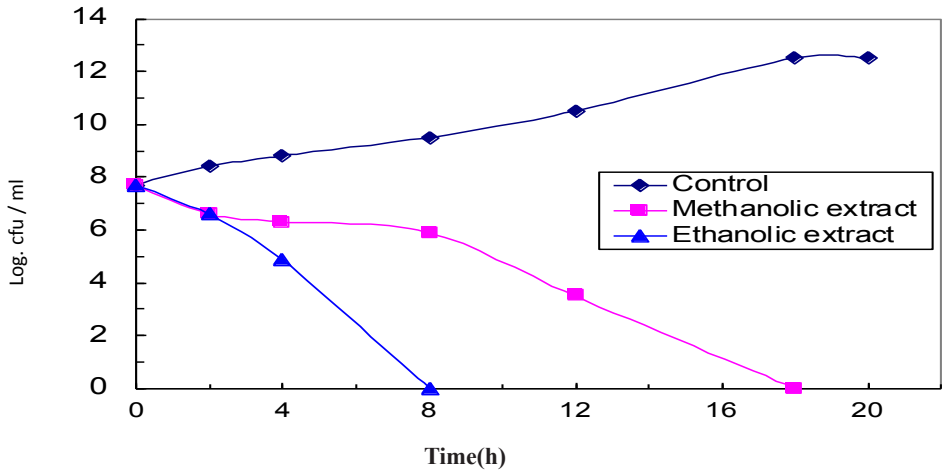

Fig. 5. Effect of ethanolic and methanolic defatted coconut meal extracts on Staphylococcus aureus subsp. aureus.

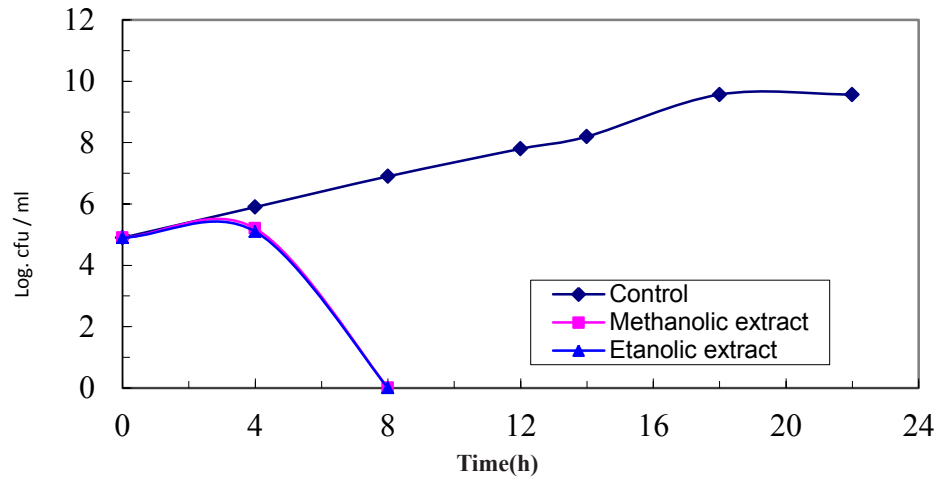

Fig. 6. Effect of ethanolic and methanolic defatted coconut meal extracts on Salmonella typhimurium.

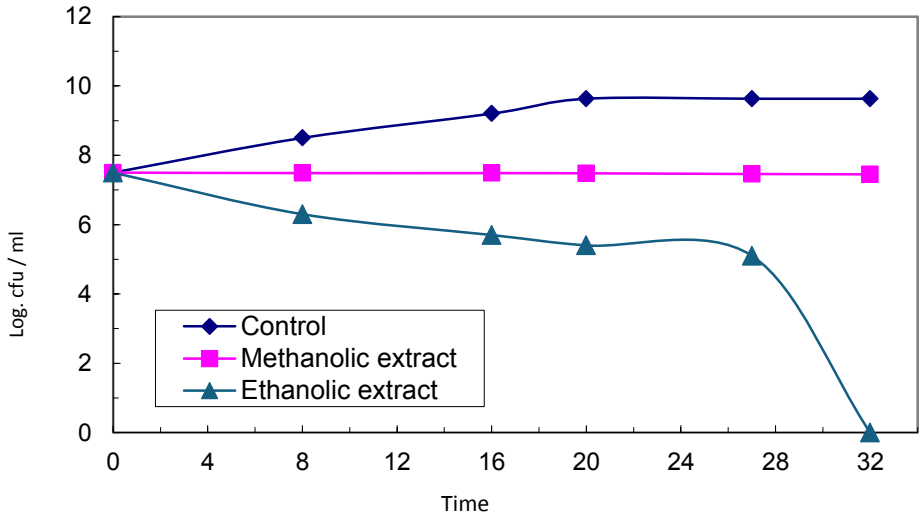

Fig. 7. Effect of ethanolic and methanolic defatted coconut meal extracts on Bacillus cereus.

inhibition effect.

Fig. (8) shows that ethanolic extract has bactericidal effect on *Bacillus stearothermophilus* after 15 h, while methanolic extract reach its bactericidal effect after 25 h of incubation.

Transmission electron microscopy bacterial strains in presence of defatted coconut meal extracts.

The ethanolic extract showed higher antibacterial activity against *Staph. aureus* subsp. *aureus* and *Salmonella typhimurium*; *B. stearothermophilus* and *B. cereus* was incubated with tested strains for 48 h at their optimum temperature, then separated by centrifugation at 10,000g and the cells were examined using TEM to determine the type of injury and the ultrastructural changes caused by the defatted coconut meal extract. The ultrastructure of the test strains without incubation with extract was examined as control. Fig.(9) illustrate a comparison of the ultrastructure of the test strains cells without and with incubation with defatted coconut meal extract as presented by TEM-micrographs. Fig. (9 a,c,e,g) depict the ultrastructure of *Staph. aureus* subsp. *aureus*, *Salmonella typhimurium*; *B. stearothermophilus* and *B. cereus* without incubation with defatted coconut meal extract as control samples. The ultrastructure of the same strains incubated with tested extract is presented in Fig. (9 b,d,f,h). Cells of *Staph. aureus* subsp. *aureus* and *Salmonella typhimurium* incubated with defatted coconut meal extract are depicted in Fig. 6b and 6d, respectively. Aggregation of cell contents and disruption the cell wall of *Staph. aureus* subsp. *aureus* can be seen. However, the observed cell deformation of *Salmonella typhimurium* (Fig. 6d) is in the form of great high dense region, which probably due to crystallization cytoplasmic proteins affecting the functionality of the cells. Also, the ribosomes cannot be seen in the deformed cells.

Fig. (9 f) shows the ultrastructure of *B. stearothermophilus* cells after incubation with defatted coconut meal extract. The characteristic structure of the gram positive cell wall cannot more be observed, probably because of a defect of cell wall biosynthesis occurred by the act of phenolic compounds present in the defatted coconut meal extract. Moreover, many transparent regions were found in the cytoplasm. The effect of defatted coconut meal extract on the cells of *B. cereus* was depicted in Fig. (9 h). Cell contents were weakly

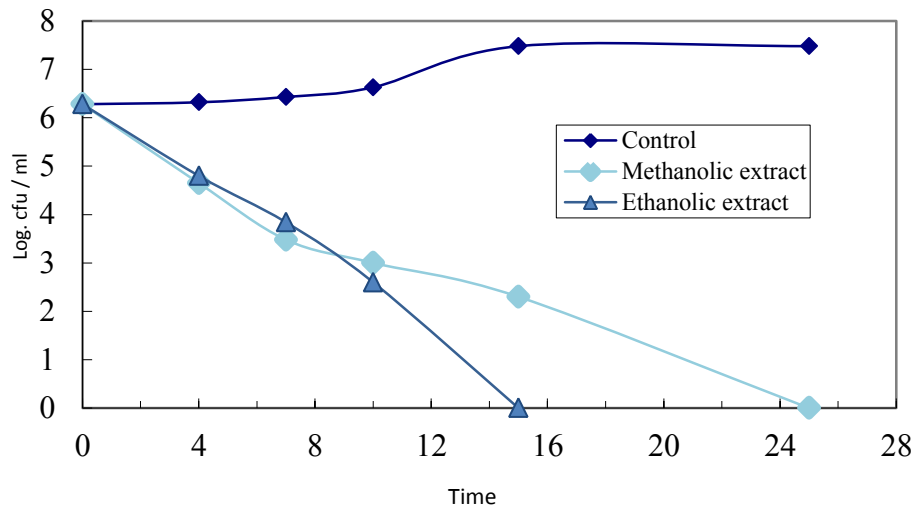

Fig. 8. Effect of ethanolic and methanolic defatted coconut meal extracts on Bacillus stearothermophilus.

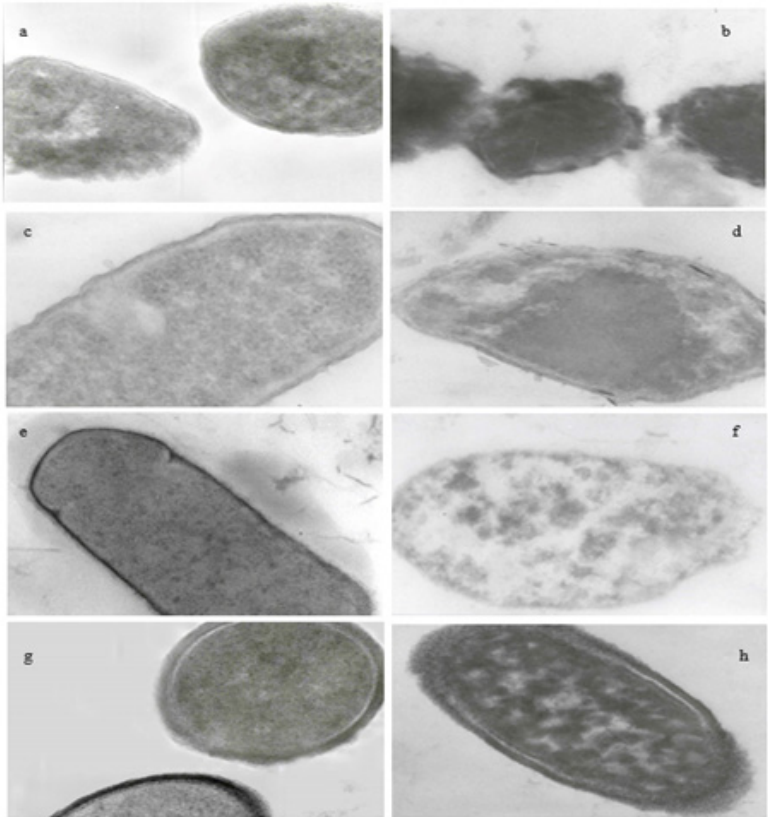

Fig. 9. Effect of defatted coconut meal extract on the ultrastructure of a gram-negative (Salmonella typhimurium) and gram-positive bacteria (Staph. aureus subsp. aureus, B. stearothermophilus and B. cereus):

(a) Staph. aureus subsp. aureus (control); (b) Staph. aureus subsp. aureus (incubated with defatted coconut meal extract); (c) Salmonella typhimurium (control); (d) Salmonella typhimurium (incubated with defatted coconut meal extract); (e) B. stearothermophilus (control); (f) B. stearothermophilus (incubated with defatted coconut meal extract); (g) B.cereus (control); (h) B.cereus (incubated with defatted coconut meal extract).

aggregated and small transparent regions formed.

Conclusion

It can be concluded that the bactericidal effect of defatted coconut meal extract most probably caused the deformation of the cells due to high content of phenolic compounds. Therefore, it can be recommended to use of defatted coconut meal or its extract as additive in the preservation of some appropriate food staffs to avoid spoilage. This is important particularly to get the benefit of the combined effect of both bactericidal activity of defatted coconut meal and thermal treatments.

References

Ahmad, R., Ahmad, N., Aljamea, A., Abuthayn, S., & Aqeel, M. (2021). Evaluation of solvent and temperature effect on green accelerated solvent extraction (ASE) and UHPLC quantification of phenolics in fresh olive fruit (Olea europaea). *Food Chemistry*, *342*, 128248.

Chen, X.; Lan, W. and Xie, J. (2024). Natural phenolic compounds: Antimicrobial properties, antimicrobial mechanisms, and potential utilization in the preservation of aquatic products. Food Chem., doi. org/10.1016/j.foodchem.2023.138198

Davidson, P.M. and Parish, M.E. (1989). Methods for testing the efficacy of food antimicrobials. Food Technol., 43, 148-155.

Pérez, M.; Dominguez-López, I. and Lamuela-Raventós, R. (2023). The Chemistry Behind the Folin–Ciocalteu Method for the Estimation of

(Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. J. Agric. and Food Chem., 71 (46) 17543-17553.

Spurr, A. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res., 26, 31-43.

Venable, J.H., and Coggeshall, R. (1965). A simplified lead citrate stain for use in electron microscopy. J. Cell. Biol., 25, 407-408.

Chen, X.; Lan, W. and Xie, J. (2024). Natural phenolic compounds: Antimicrobial properties, antimicrobial mechanisms, and potential utilization in the preservation of aquatic products. Food Chem., doi.org/10.1016/j.foodchem.2023.138198

Pietrzak, W.; Nowak, R.; , Gawlik-Dziki, U.; Lemieszek, M. K. and Rzeski, W. (2017). LC-ESI-MS/MS Identification of Biologically Active Phenolic Compounds in Mistletoe Berry Extracts from Different Host Trees. Molecules,12;22(4):624. doi: 10.3390/molecules22040624.

Trinidad, T.; Mallillin, A.C.; Valdez, D.H.; Loyola, A.S.; Askali-Mercado, F.C.; Castillo, J.C.; Encabo, R.R.; Masa, D.B.; Maglaya, A.S.; and Chua, M.T. (2006). Dietary fiber from coconut flour: a functional food. Innovat. Food Sci. Emerg. Technol., 7 (4) 309-317, 10.1016/j.ifset.2004.04.003.

Adekola, K.A.; Salleh, A.B.; Zaidan, U.H.; Azlan, A.; Chiavaro, E.; Paciulli, M. Total phenolic Content, Antioxidative and Antidiabetic Properties of Coconut (Cocos Nucifera L.) Testa and Selected Bean Seed Coats. Ital. J. Food Sci. 2017, 29, 741–753.

Akhter, A.; Zaman, S.; Ali, U.; Ali, Y.; Miah, M.A.J. Isolation of Polyphenolic Compounds from the Green Coconut (Cocos Nucifera) Shell and Characterization of Their Benzoyl Ester Derivatives. J. Sci. Res. 2009, 2, 186–190.

Appaiah, P.; L., S.; A. G., G.K.; G., S.K. Phytochemicals and Antioxidant Activity of Testa Extracts of Commercial Wet and Dry Coconuts and Cakes. Int. Res. J. Pharm. 2016, 7, 9–13.

Arivalagan, M.; Roy, T.K.; Yasmeen, A.M.; Pavithra, K.C.; Jwala, P.N.; Shivasankara, K.S.; Manikantan, M.R.; Hebbar, K.B.; Kanade, S.R. Extraction of Phenolic Compounds with Antioxidant Potential from Coconut (Cocos Nucifera L.) Testa and Identification of Phenolic Acids and Flavonoids Using UPLC Coupled with TQD-MS/MS. LWT-Food Sci. Technol. 2018, 92, 116–126.

Ojha, S.; Roy, S. and Dhangadamajhi, G. (2019). Phytochemicals Screening, Phenolic Estimation and Evaluation for Antioxidant, Anti-Inflammatory and Anti-Microbial Activities of Sequentially Soxhlet Extracted Coconut Testa. Food and Nutrition Sciences.

Rambabu, K.; Bharath, G.; Banat, A.H.F.; Hasan, S.W. Taher, H. and Zaid, H.F.M. (2020). Nutritional quality and physico-chemical characteristics of selected date fruit varieties of the United Arab Amirates, Processes, 8, 256.

Nehdi, I.; Omri, S.; Khalil, M.I. and Al-Resayes, S.I. (2010). Characteristics and chemical composition of date palm (Phoenix canariensis) seeds and seed oil. Industrial Crops and Products, 32, 360-365.

Akasha, I.; Campbell, L.; Lonchamp, J. and Euston S.R. (2016). The major proteins of the seed of the fruit of the date palm (*Phoenix dactylifera* L.): Characterisation and emulsifying properties. Food Chem., 15:197,799-806.

Schoenknecht, F. D.; Sabath, L.D. and Thornsberry, C. (1995). Susceptibility tests, special tests. In "Manual of clinical Microbiology" ed. Lennette E, 4th ed., pp.1000-1001, Am. Soc. for Microbiology, Washington, DC.