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Lactic acid bacteria fermentation of coconut milk and its effect on the nutritional, phytochemical, antibacterial and sensory properties of virgin coconut oil produced

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Coconut oil has profound health benefits but the high content of fatty acids is a concern to many consumers, as several processing methods have failed to produce oil with considerable change in fatty acid content. In this study, selected lactic acid bacteria including *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Leuconostoc mesenteroides* and *Enterococcus faecium* were used to ferment coconut milk for production of virgin coconut oil. Fermentation was carried out for 48 h, after which the milk was processed through heating to produce coconut oil. Phytochemical content, proximate composition, determination of steroids and free fatty acid content of the oil and antibacterial activity of the oil against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were carried out using standard analytical and laboratory procedures. The results obtained in this study indicate that the control and LAB fermented oil had similar composition of phytochemicals; steroids, anthraquinone and glycosides were present in all the virgin coconut oil produced; however, reduced moisture content, lower levels of ester and free fatty acid was observed and higher quantity of protein and ash were obtained for coconut oil fermented with LAB compared to the control. The virgin coconut oil produced using the starter culture also had higher acceptability ($P<0.05$) compared to the spontaneously fermented oil, as the milk fermented with *Lactobacillus plantarum* had the highest acceptability value. There was no significant difference in the antibacterial activity of the virgin coconut oil produced against the test organisms. This study indicates that free fatty acid and other undesirable constituents in coconut oil can be reduced using lactic acid bacteria as starter culture, hence, increasing the acceptability of the product.

Key words: Lactic acid bacteria, coconut milk, fermentation, coconut oil.

INTRODUCTION

Coconut oil is extensively used for food, industrial applications, health promotion and disease prevention (Manisha and Shyamapada, 2011). Natural fermentation is a method where less processing conditions are involved. In the natural fermentation process, extracted milk from wet coconut was allowed for microbial

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fermentation (Divina, 2002). Lactic acid bacteria (LAB) for a long time have been applied as starter cultures in fermented foods and beverages because they can improve nutritional, organoleptic, technological and shelf-life characteristics. LAB can also produce ethanol, bacteriocins, aroma components, exopolysaccharides and some enzymes (De Vuyst and Leroy, 2004).

Lactic acid bacteria play a key role in the VCO fermentations where they not only contribute to the development of the desired sensory properties in the final product but also to their microbiological safety. Fermentation is one of the most ancient and most important food processing technologies. Fermentation is a relatively efficient, low energy preservation process, which increases the shelf life and decreases the need for refrigeration or other forms of food preservation technology. LABs are considered Generally Recognized as Safe (GRAS). LABs produce antimicrobial agents including organic acids, hydrogen peroxides and bacteriocin. LAB has been known for many years and play important role in the production of a variety of fermented foods. Health benefits of LAB are known to give positive influence in the gastrointestinal of humans (Hafidh et al., 2010). Lactic Acid Bacteria (LAB) constitute part of the autochibonous microbiota of many types of foods. They are defined as a clutter of lactic acid-producing Low G+C%, non-spore forming, Gram-positive rods and cocci and catalase-negative which share many bio-chemical, physiological and genetic properties (Abriouel et al., 2004). This group of bacteria has a particular interest for food industries due to their technological properties, being often used as starter cultures to produce fermented products.Virgin coconut oil (VCO) seemed to be the purest form of coconut oil, water white in color, contains natural vitamin E and with very low free fatty acid content and low peroxide value. It has a mild to intense fresh coconut aroma. VCO may be defined as the naturally processed (Nour et al., 2009), chemically-free and additive-free product from fresh coconut meat or its derivative (coconut milk and coconut milk residue) which has not undergone any further chemical processing after extraction (Marina et al., 2009).

This study therefore aimed at using selected lactic acid bacteria as starter culture for fermentation of coconut milk and evaluating the effect on quality attributes of the coconut oil produced.

MATERIALS AND METHODS

Coconut sample

Randomly selected uniformly sized, 12 months old (matured) nuts were collected from local commercial market.

Source of indicator organisms

Staphylococcus aureus and *Pseudomonas aeruginosa* were isolated from spoilt coconut fruits.

Microbial culture

Pure culture of five lactic acid bacteria isolated from fresh fruits possessing antibacterial activity and safe to be used as probiotics were used as seed culture in different vessels; one milliliter of 10^5MacFarland of each of the bacteria was introduced into 250 ml of coconut milk for fermentation.

Coconut milk extraction

Coconuts were broken and solid endosperm was collected, testa was removed by using kitchen peeler, white coconut balls were disintegrated into small pieces and grinded with 1:2 ratio of water for 10 min. Ground mass was transferred to the muslin cloth, pressed manually for coconut milk extraction; the same process was repeated twice and coconut milk was pooled up.

Phytochemical and physicochemical composition

The saponification, iodine and acid value were determined according to the titre metric method of Pearson (1981) while peroxide value was evaluated according to AOAC (1984).

Proximate composition

Proximate composition of the virgin coconut oil: Moisture content, ash, carbohydrate, crude protein, fat and crude fiber were determined according to AOAC (1984).

Determination of antibacterial activity

The indicator organisms were streaked on sterile molten Mueller Hinton agar, paper discs were impregnated with oil and Vancomycin paper disc was used as control following the method of Nguyen et al. (2017).

Statistical analysis

The means of the results were evaluated using analysis of variance (ANOVA), and the Tukey test was used to compare differences ($p < 0.05$) among the technological, physicochemical, microbiological and sensory evaluations. Statistical Analysis System (SAS, 1999) was used.

RESULTS AND DISCUSSION

Phytochemical screening of the virgin coconut oil (Table 1) reveals that all the samples contain steroids, terpenoids, cardiac glycosides while alkaloids, flavonoids, saponins and tannins were absent. Ghosh et al. (2014) detected terpenoid and steroids in virgin coconut oil and their report is similar to our study; however, tannins were present in their oils contrary to our observation in this study. The presence of these phytochemicals in coconut oil vary as different researchers differ in their reports.

Though there is dearth of information on induced

Table 1. Phytochemical composition of coconut oil produced using lactic acid bacteria starter.

S/N	Starter fermented samples	Alkaloids	Flavonoids	Saponins	Tannins	Anthraquinnones	Terpenoids	Steroids	Cardiac glycosides
1	IB	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
2	C_{19}	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
3	C_{17}	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
4	C_{26}	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
5	C_{12}	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
6	CCO	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve

H: Hot; C=Cold; -ve= Negative; +ve =positive; MC=Moisture content; CP=Crude protein; Crude Fiber; CCO= Control; IB=sample fermented with *Leuconostoc mesenteroides*; C_{12} = sample fermented with *Lactobacillus plantarum*in hot water extraction; C_{17}= sample fermented with *L. pentosus*; C_{19}= sample fermented with *L. plantarum*in cold water extraction; C_{26}= samples fermented with *Enterococcus faecium*.

Table 2. Physicochemical quality of coconut oil produced using lactic acid bacteria starter.

Starter fermented samples	Saponification value	Iodine value	Ester value
IB	444.6	19.98	424.62
C_{19}	402.9	20.62	382.28
C_{26}	468.4	19.99	434.62
C_{12}	420.2	20. 53	387.28
C_{17}	403.4	20. 42	384.28
CCO	472. 4	20.62	451.78

H: Hot; C=Cold; MC=Moisture content; CP=Crude protein; Crude fiber; CCO= Control; IB=sample fermented with *Leuconostoc mesenteroides*; C_{12} = sample fermented with *Lactobacillus plantarum* in hot water extraction; C_{17}= sample fermented with *.pentosus*; C_{19}= sample fermented with *L. plantarum*in cold water extraction; C_{26}= samples fermented with *Enterococcus faecium.*

Table 3. Physical parameters of coconut oil produced using lactic acid bacteria starter.

S/N	Starter fermented samples	Refractive index	pH value	Specific gravity
1	IB	1.654	3.86	0.9130
2	C_{19}	1.657	3.28	0.9140
3	C_{17}	1.654	3.42	0.0.925
4	C_{26}	1.655	3.43	0.8990
5	C_{12}	1.660	3.26	0.8890
6	CCO	1.652	3.69	0.9230

CCO= Control; IB=sample fermented with*Leuconostoc mesenteroides*; C_{12} = sample fermented with *Lactobacillus plantarum*in hot water extraction; C_{17}= sample fermented with *L. pentosus*; C_{19}= sample fermented with *L. plantarum* in cold water extraction; C_{26}= samples fermented with *Enterococcus faecium.*

fermentation of coconut milk by lactic acid bacteria, the result obtained in this study as regards the physicochemical quality of the oil meets international codex standard and agrees with the report of Satheesh and Prasad (2012) that produced coconut oil using lyophilized culture of *Lactobacillus plantarum.* The moisture content of the coconut oil produced in this study ranges between 0.11 to 0.40; the international standard is 0.1 -0.5, all the LAB strains used produce coconut oil with very low moisture content (0.11 -0.20), and it is believed that moisture plays a major role in the shelf life of oils as it influences its rancidity.Our report is not similar to what was reported by Satheesh and Prasad (2012) that used innovative wet process for production of coconut oil. Physiochemical property of the coconut oil (Table 2) such as iodine value and ester value ranges between 382.28 to 451.78, the refractive index ranges between 1.65 to 1.66, the specific gravity is between 0.89 to 0.93 and pH values ranged from 3.26 to 3.86 (Table 3). Further, a significant difference exist between the specific gravity of virgin coconut oil produced using *L. plantarum* along with hot and cold extraction method, as the result of the quality parameters of the VCO produced in this study meets quality standard and is similar to earlier report of

Table 4. Proximate composition of coconut oil produced using lactic acid bacteria starter.

S/N	Sample ID	% MC	% CP	%CF	%Ash
1	CCO	0.30	0.71	98.91	0.09
2	IB	0.21	1.08	98.61	0.21
3	C_{12}	0.29	1.75	97.85	0.30
4	C_{17}	0.20	1.35	98.46	0.20
5	C_{19}	0.13	1.03	98.71	0.13
6	C_{26}	0.39	1.06	97.61	0.20

MC=Moisture content; CP=Crude protein; Crude Fiber; CCO = Control; IB=sample fermented with *Leuconostoc mesenteroides*; C_{12} = sample fermented with *Lactobacillus plantarum* in hot water extraction; C_{17} = sample fermented with *L. pentosus*; C_{19} = sample fermented with *L. plantarum* in cold water extraction; C_{26} = samples fermented with *Enterococcus faecium*.

Table 5. Sensory evaluation of starter fermented coconut oil.

Sample	Taste	Aroma	Colour	Flavor	Overall acceptability
CCO	4.4^{a} ±0.50	4.0^{a}±0.99	4.55^{b}±0.76	4.35^{a}±0.49	4.33^{a} ±0.50
IB	4.05^{a}±0.83	4.10^{a}±0.91	4.75^{ab}±0.55	4.60^{a}±0.50	4.49^{a} ±0.75
C_{12}	4.05^{a}±0.95	4.05^{a}±0.95	4.70^{ab}±0.66	4.55^{a}±0.51	4.32^{a} ±0.51
C_{17}	4.00^{a}±0.97	4.30^{a}±0.73	4.80^{ab}±0.37	4.55^{a}±0.51	4.40^{a} ±0.85
C_{19}	4.30^{a}±0.73	4.25^{a}±0.85	4.85^{ab}±0.37	4.55^{a}±0.51	4.41^{a}±0.76
C_{26}	4.00^{a}±0.65	4.00^{a}±0.80	4.75^{ab}±0.44	4.75^{ab}±0.44	4.30a±0.54

The values represent mean± standard deviation. Also, values with the same superscript are not significantly different; CCO=control spontaneous fermentation.

Table 6. Antibacterial activity of starter fermented coconut oil against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Sample	*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
	Zone of inhibition (mm)	Vancomycin	Zone of inhibition (mm)	Vancomycin
C_{12}	4	5	4	5
C_{17}	2.5	5	3	5
C_{26}	4	4	2.5	7
IB	2	4	3	5
C_{19}	3	5	5	6
CCO	3	4	2	5

CCO= Control; IB=samples fermented with *Leuconostoc mesenteroides*; C_{12} = samples fermented with *Lactobacillus plantarum* in hot water extraction; C_{17}= samples fermented with *L. pentosus*; C_{19}= samples fermented with *L. plantarum* in cold water extraction; C_{26}= samples fermented with *Enterococcus faecium.*

Thanuja et al. (2016).

Quantitative determination of steroids in the virgin coconut oil produced shows that the control has high percentage of steroids and free fatty acid compared to the oil fermented with specific starter culture of selected. There is significant difference.in the quantity of protein present in LAB fermented coconut milk (1.07 to 1.76) and spontaneously fermented coconut (1.06) for coconut oil production. Higher protein content was obtained compared to the control samples; and the moisture content of the oil was lower in all LAB produced coconut oil except the oil produced using *Leuconostoc mesenteroides*, reduced moisture and ash content compared to the control (Table 4).

All the oil samples have similar general acceptability in terms of flavor and color. Oil produced using *L. plantarum* had a higher acceptability in terms of taste while the aroma of the oil produced with *L. mesenteroides* had the highest acceptability (Table 5). The zone of inhibition produced by using coconut oil in agar well diffusion method as antibacterial against *S. aureus* (Table 6) is similar to the standard antibiotics.

Conclusions

Based on our findings, it can therefore be said that fermentation of coconut milk with LAB as starters for

production of virgin coconut oil (VCO) be encouraged as the VCO produced had better nutritive and sensory properties, a prominent attribute of foods fermented with lactic acid bacteria. Also, the fatty acid and moisture content of the oil produced is significantly lower compared to the control, a property most desired in edible oils.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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