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ORIGINAL ARTICLE

Application of Polyphenol Extract from Mangosteen Pericarp for Milk Powder Preservation

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ABSTRACT
Phenolic compounds are raising great interest in medical and scientific research for their health benefits, which include anti-carcinogenic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-hypertensive activities. In this research, we focus on selecting the proper polyphenol extract from mangosteen pericarp supplementing to milk powder to extend its shelf-life through peroxide value. Moreover we also examine milk powder characteristics such as total polyphenol, total flavonoid, anti-oxidation capability by DPPH and reduction by iron, and other criteria of milk powders such as flowing, wetting, solubility, density, free lipid, emulsification and micro-encapsulation. Our results show that milk powder shelf-life in 733 days with formula 30% defatted milk, 10% soybean, 0.5% lecithin, 5.2% maltodextrin, 2% lactose, 0.3% mangosteen pericarp and 52% water. Total polyphenol and anti-oxidation capability (IC_{50}) after being spray drying are 281.1 mgGAE/100g milk powder and 13.63 ppm. Protein 13.9%, lipid 20.27%, flavonoid 6.1 mgRE/100g milk powder, anti-oxidation capability by DPPH 13.63 ppm, reduction by iron 0.54 mg, vitamin C/l, micro-encapsulation 84.42%, emulsification 91.29%.
Keywords: *Polyphenol, mangosteen, milk powder, shelf-life, preservation*

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INTRODUCTION

Phenolic compounds are one of the most represented groups of substances in the plant kingdom. Until now, the polyphenols in green tea, black tea, grape and wine (especially red) have been extensively studied and characterized. The mangosteen (*Garcinia mangostana*) is a tropical fruit native to Southeast Asia and has long been reported to contain multiple health promoting properties. This fruit is an abundant source of xanthones, a class of polyphenolic compounds with a distinctive tricyclic aromatic ring system and is largely responsible for its biological activities including anti-cancer activity. The major bioactive compounds found in mangosteens are phenolic acid, prenylated xanthone derivatives, anthocyanins, and procyanidins [2, 5, 6, 17]. Protocatechuic acid was the major phenolic acid in the peel and rind, while p-hydroxybenzoic acid was the predominant phenolic acid in the aril [17]. The major anthocyanin in mangosteen was cyanidin-3-sophoroside [5]. Several researchers recognized phenolics and anthocyanin for their antioxidant properties [1, 4, 9, 15, 16].

There are several sesearch mentioned to polyphenol extraction such as:

Dried ground leaves of Psidium guajava L. (guava) were extracted by water and aqueous ethyl alcohol 50% (1:10) ratio, and the total phenolic content in the extracts was determined spectrophotometrically according to Folin Ciocalteu's phenol method and calculated as gallic acid equivalent (GAE) [14]. The active component of the aqueous guava leaf extract and its inhibition of alpha-glucosidase enzymes *in vitro*, safety of the extract and Guava Leaf Tea, reduction of postprandial blood glucose elevation, and improvement of hyperglycemia, hyperinsulinemia, hypoadiponectinemia, hypertriglycemia and hypercholesterolemia in murine models and several clinical trials. It is suggested that the chronic suppression of postprandial blood glucose elevation is important in preventing type 2 diabetes mellitus, and that Guava Leaf Tea is considered useful as an alimentotherapy for chronic treatment [18]. Research has been conducted to determine the levels of tannins in leaves of guava (Psidium guajava L) using a variation of the concentration of organic solvent. The method used for qualitative analysis with the tannins are formed by the intensity of the color is blackish green FeCl3 compounds [11]. The molecular interaction of cocoa polyphenols with milk proteins were investigated in vitro by combined proteomic and

biochemical strategies. Mass spectrometry and antioxidant activity assays allowed monitoring the binding of casein and whey protein fractions to cocoa polyphenols [13]. They examined the antibacterial activity of extract from mangosteen pericarp against *Streptococcus* mutans, bacteria associated with dental plaque formation and caries development [10]. They compared the antioxidant activity of the peel and pulp extracts of Garcinia tinctoria (yellow mangosteen) fruits [12]. Total phenolic content (TPC) assay showed that the peels contained higher phenolic content than the pulps [3].

The study focuses on the anthocyanin and total phenolic content of mangosteen, the effect of drying on the quality of mangosteen mixed with fruit juice powder, and the effect of enzyme clarification and evaporation methods on the quality of mangosteen concentrate such as color value, anthoycanin and total phenolic content, and the percent of polymeric color [3].

Based on the reported findings there is clear evidence that these polyphenols target multiple signaling pathways involved in cell cycle modulation and apoptosis. Further work is required to understand its potential for health promotion and potential drug discovery for prostate and breast cancer chemoprevention [7].

The main purpose of this research is to investigate the proper polyphenol extract from mangosteen pericarp supplementing to milk powder to extend its shelf-life through peroxide value. Moreover we also examine milk powder characteristics such as total polyphenol, total flavonoid, anti-oxidation capability by DPPH and reduction by iron, and other criteria of milk powders such as flowing, wetting, solubility, density, free lipid, emulsification and micro-encapsulation.

MATERIAL AND METHOD

Material

We collect mangosteen fruit in Mekong River Delta, Vietnam. Then we separate pericarp and pulp to get pericarp.

Figure 1. Mangosteen fruit

Research method

Weigh 10g into the beaker 2 L, ethanol 50% with ratio of material: solvent 1:25. Treat with microwave at 385W in 13 minutes. The extract compound is filtered by vaccuming. Evaporate the solvent at 45^{o}C in 15 minutes to get the extract. Keep it in cool and dry condition. Defatted milk, maltodextrin, lactose are mixed with water at 50-60^{o}C, soybean oil and lecithin. The polyphenol extract and FeSO4 are supplemented at the final step. After mixing, the compound is then homogenized at 200 bar. The homogenized fluid is heated at 72 – 75°C in 15 –20 seconds. Keep a minor portion to check physic-chemical characteristics: total polyphenol, total flavonoid, anti-oxidation capability by DPPH and reduction by iron. The remaining portion is dried by spraying Lab Plant (England, SD-06AG) temperature 80^{o}C, pressure 3 bar, pumping 695 ml/h.

Statistical analysis

All data are processed by ANOVA.

RESULT AND DISCUSSION

Total polyphenol, flavonoid, anti-oxidation capability by DPPH of milk supplemented with mangosteen pericarp extract before and after spray drying.

Total polyphenol TPC) and total flavonoid (TFC)

We investigate the milk powder supplemented with different polyphenol extract: CT1, CT2, CT3 equivalent to 0.2 %, 0.3% and 0.5%.

Table 1. Total polyphenol and flavonoid before and after spray drying

Sample	Total polyphenol (mgGAE/100gDW)		Total flavoid (mgRE/100g)	
	Before spray drying	After spray drying	Before spray drying	After spray drying
Control	0	0	0	0
0.2% extract	$284.6^{a} \pm 1.3$	$225.8^{a} \pm 0.6$	$8.2^{x} \pm 0.1$	$5.3^{x} \pm 0.1$
0.3% extract	$380.1^{b} \pm 2.7$	$281.1^{b} \pm 2.3$	$8.9^{y} \pm 0.1$	$6.1^{y} \pm 0.2$
0.5% extract	$619.6^{c} \pm 4.1$	$458.9^{c} \pm 4.0$	$16.8^{z} \pm 0.3$	$10.4^{z} \pm 0.1$

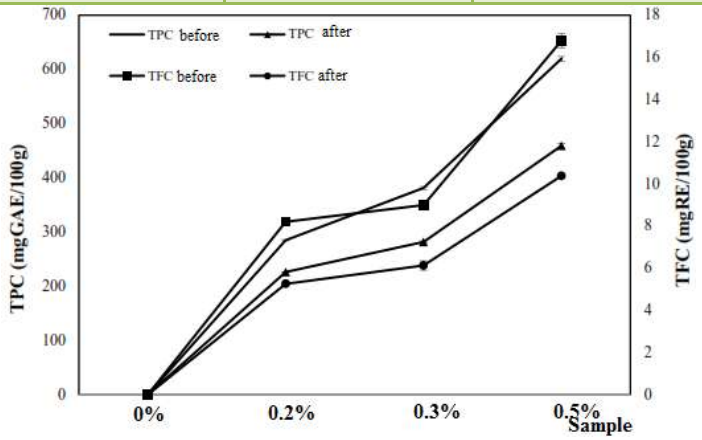

Figure 3. Total polyphenol and total flavonoid before and after spray drying

We choose 0.2% extract to supplement into milk powder to get TPC 232.2 mgGAE/100g.

Anti-oxidation capability by DPPH and iron reduction

Table 2. Anti-oxidation capability by DPPH and iron reduction

Sample	DPPH IC_{50} (ppm)		Iron reduction (mg Vitamin C/l)	
	Before spray drying	After spray drying	Before spray drying	After spray drying
Control	0	0	0	0
0.2% extract	$10.72^{a} \pm 0.27$	$15.4^{a} \pm 0.14$	$0.69^{x} \pm 0.22$	$0.42^{x} \pm 0.001$
0.3% extract	$9.68^{b} \pm 0.57$	$13.63^{b} \pm 0.1$	$0.79^{y} \pm 0.02$	$0.54^{y} \pm 0.010$
0.5% extract	$7.07^{c} \pm 0.61$	$9.73^{c} \pm 0.04$	$0.99^{z} \pm 0.06$	$0.79^{z} \pm 0.003$

From above result we see the anti-oxidation capability decrease CT3>CT2>CT1 and the sample before spray drying > after spray drying for both DPPH and iron reduction.

Effect of extract to milk powder characteristics

Moisture and ash of milk powder

Table 3. Moisture and ash of milk powder by different polyphenol extract

Sample	Moisture (%)	Ash (%)
0% (control)	$2.93^{a} \pm 0.03$	$2.64^{a} \pm 0.01$
0.2% polyphenol extract	$3.69^{b} \pm 0.06$	$2.66^{b} \pm 0.01$
0.3% polyphenol extract	$3.92^{c} \pm 0.03$	$2.67^{c} \pm 0.01$
0.5% polyphenol extract	$4.18^{d} \pm 0.04$	$2.68^{d} \pm 0.01$

Bulk density

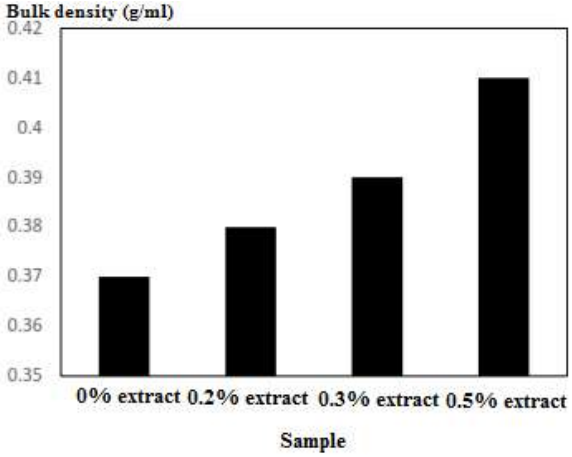

Figure 3. Bulk density of milk powder by different polyphenol extract

Table 4. Bulk density of milk powder by different polyphenol extract

Sample	Density (g/l)
0% (control)	$0.37^{a} \pm 0.01$
0.2% polyphenol extract	$0.38^{b} \pm 0.02$
0.3% polyphenol extract	$0.39^{c} \pm 0.11$
0.5% polyphenol extract	$0.41^{d} \pm 0.09$

Flowing/ melting of milk powder

Table 5. Flowing/ melting of milk powder by different polyphenol extract

Sample	0% extract	0.2% extract	0.3% extract	0.5% extract
Flowing angle α°	$49.7^{a} \pm 0.6$	$48.1^{b} \pm 1.7$	$50.0^{c} \pm 1.8$	$53.0^{d} \pm 1.0$

Solubility of milk powder

Table 6. Solublitity of milk powder by different polyphenol extract

Sample	0% extract	0.2% extract	0.3% extract	0.5% extract
Solubility (%)	$93.1^{a} \pm 0.4$	$96.5^{b} \pm 0.4$	$99.6^{c} \pm 0.4$	$92.0^{d} \pm 0.1$

We see quite clearly that sample treated 0.3% polyphenol extract; we get the milk powder solubility 996%. After that polyphenol extract 0.2%, solubility 96.5%. Meanwhile, the control sample has solubility 93.1%.

Wetting time

All samples treated 0%, 0.2%, 0.3% and 0.5% polyphenol extract have the wetting time 300 seconds. This phenomenon is not good for milk powder.

Lipid content, micro-encapsulation and emulsification

Table 7. Lipid content, micro-encapsulation and emulsification

Sample	0% extract	0.2% extract	0.3% extract	0.5% extract
Total lipid (%)	20.65 ± 0.8	19.87 ± 0.7	20.27 ± 1.1	19.68 ± 0.7
Micro-encapsulation (%)	85.18 ± 0.4	83.74 ± 0.6	84.42 ± 1.2	77.57 ± 1.9
Emulsification (%)	95.57 ± 2.4	91.59 ± 3.0	91.29 ± 1.6	87.72 ± 3.1

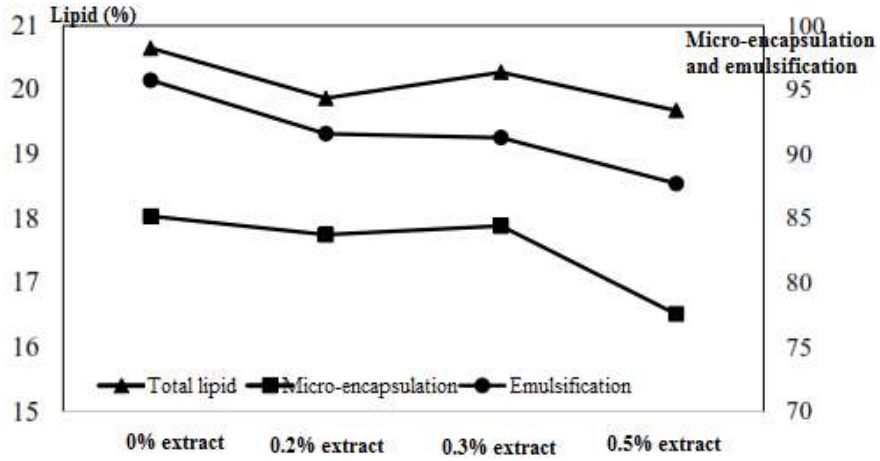

Figure 4. Lipid content, micro-encapsulation and emulsification of milk powder

Protein

Table 8. Protein in milk powder

Sample	Protein (%, w/w) before spray drying	Protein (%, w/w) after spray drying
0% (control)	$16.9^{a} \pm 0.16$	$14.8^{a} \pm 0.22$
0.2% polyphenol extract	$16.2^{b} \pm 0.16$	$14.3^{b} \pm 0.10$
0.3% polyphenol extract	$15.6^{c} \pm 0.24$	$13.9^{c} \pm 0.22$
0.5% polyphenol extract	$14.4^{d} \pm 0.43$	$13.3^{d} \pm 0.36$

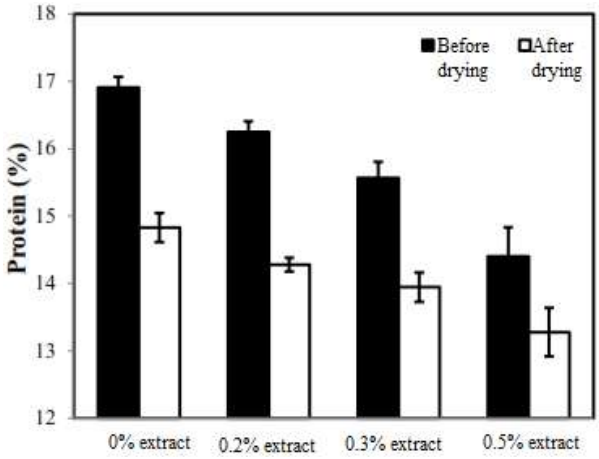

Figure 5. Protein in milk powder before and after spray drying

After spray drying, the protein remaining in milk powder samples treated by 0%, 0.2%, 0.3%, 0.5% polyphenol extract are 12.4%, 11.7%, 10.9%, 7.6% in equivalent.

Acidity in milk powder

Table 9. Acidity in milk powder

Sample	Protein (%, w/w) before spray drying	Protein (%, w/w) after spray drying
0% (control)	$0.39^{a} \pm 0.04$	$0.40^{a} \pm 0.06$
0.2% polyphenol extract	$0.48^{b} \pm 0.03$	$0.49^{b} \pm 0.04$
0.3% polyphenol extract	$0.53^{bc} \pm 0.05$	$0.54^{bc} \pm 0.06$
0.5% polyphenol extract	$0.58^{c} \pm 0.05$	$0.61^{c} \pm 0.05$

SEM photograph

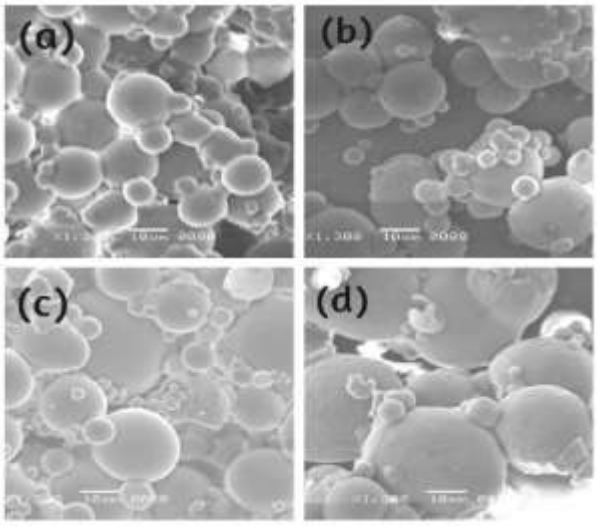

Figure 6. SEM of milk powder:
(a) 0% extract; (b) 0.2% extract; (c) 0.3% extract; (d) 0.5% extract

We see quite clearly that the sample treated with 0.3% polyphenol extract has the best flowing, wetting capability compared to other samples.

Color measurement by Minota

Table 10. Color of milk powder L*, a*, b*, C* H* and ΔE*

Sample	L*	a*	b*	c*	H*	ΔE*
0% (control)	96.41 ± 0.15	-2.05 ± 0.11	13.79 ± 0.39	13.94 ± 0.39	-81.55 ± 0.23	10.13 ± 0.36
0.2% polyphenol extract	99.34 ± 0.03	-2.27 ± 0.02	13.32 ± 0.14	13.51 ± 0.12	-80.34 ± 0.04	10.75 ± 0.14
0.3% polyphenol extract	98.40 ± 0.01	-2.26 ± 0.03	12.70 ± 0.01	12.90 ± 0.01	-79.93 ± 0.14	9.79 ± 0.01
0.5% polyphenol extract	94.48 ± 0.54	-1.27 ± 0.15	20.80 ± 0.72	20.84 ± 0.71	-86.49 ± 0.49	16.81 ± 0.71

Effecrt of polyphenol extract to milk powder shelf-life

Peroxide value during preservation

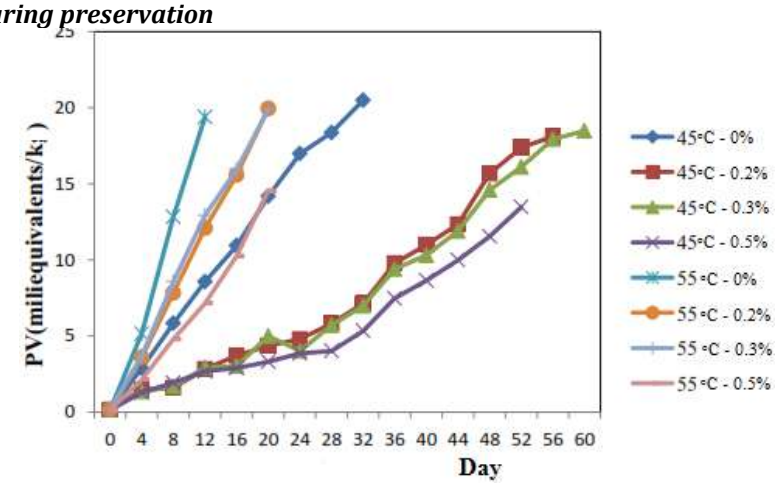

Figure 7. Peroxide value during preservation

Milk powder shelf-life

Table 12. Milk powder shelf-life affected by polyphenol extract

Sample	K (45°C)	R^2 (45°C)	K (55°C)	R^2 (55°C)	F25°C	Ea	PV at the end of shelf life	
							45°C	55°C
0% extract	0.6382	0.9979	1.7787	0.9927	260	10.53	20.489	19.38
0.2% extract	0.338	0.9529	1.0722	0.9855	638	0.51	18.108	20.035
0.3% extract	0.2438	0.9248	0.6361	0.018	733	0.47	18.472	19.88
0.5% extract	0.2438	0.9248	0.6361	0.018	360	2.65	13.473	15.27

From table above, we notice the difference of product shelf-life among samples. The control sample (0% extract) has the lowest shelf-life.

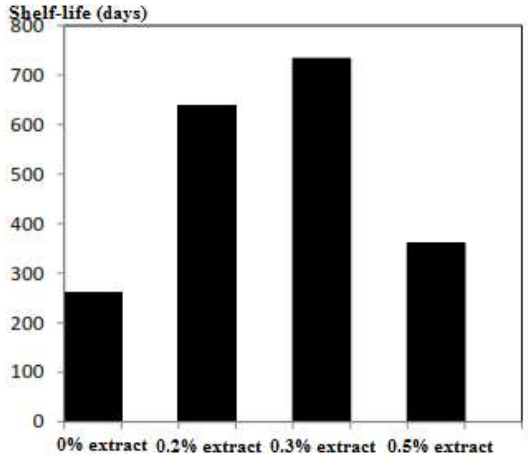

Figure 8. Milk powder shelf life affected by polyphenol extract

CONCLUSION

Mangosteen (*Garcinia mangostana* Linn) is a tropical fruit in Guttiferae family. Mangosteen is dark purple to red-purple fruits. The edible fruit aril is white, soft, and juicy with a sweet, slightly acid taste and a pleasant aroma. We have successfully defined the mixing formula to extend product shelf-life with 0.3% extract, 733 days (45°C).

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