

# Potency of Bioactive Compound of Rice Bran for Colon Cancer Prevention

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**Submission date:** 18-Sep-2019 08:23AM (UTC+0700)

**Submission ID:** 1174796115

**File name:** Review\_Article-Safrida-IPN\_2017\_turnitin.docx (156.14K)

**Word count:** 3425

**Character count:** 19291

# Potency of Bioactive Compound of Rice Bran for Colon Cancer Prevention

## Abstract

Colon cancer is the second leading cause of death in the world. Bioactive compounds in rice bran have a very active role as antiproliferation of colon cancer cells such as ferulic acid, p-coumaric acid, caffeic acid, gallic acid, protocatechuic acid, sinapic acid, triclin, luteolin, apigenin, myricetin, rutin, isorhamnetin,  $\gamma$ -oryzanol,  $\gamma$ -tocopherol,  $\delta$ -tocopherol,  $\gamma$ -tocotrienol,  $\beta$ -sitosterol, phytic acid, and hemicellulose. Mechanism of the bioactive compounds in cells varied, including modulation of a cell cycle, activation of immune cells, damage of a lipid layer and mitochondrial membrane, activation of caspase proteins, inhibition of protein cell tumor invasion, metastasis, and angiogenesis, and also acts as an antioxidant. Therefore, the existence of the scientific studies results of this review with the potential availability of adequate rice bran in Indonesia is very potential to be developed.

Keywords: Rice bran, Bioactive compound, Colon cancer

## 1. Introduction

Bioactive compounds are phytochemicals that can be found in food, serves to modulate metabolic processes to improve health. The bioactive compounds in rice bran have been widely known that have a role in reducing several diseases such as hyperlipidemia (1), antiproliferation in cancer cells (2,3,4,5), antidiabetic (6,7,8) chronic kidney disease and acute coronary syndrome (9).

Rice bran is a by-product of the rice milling process. In the process of rice milling, 10% of rice bran will be produced. The potential of rice bran that produced was estimated at 5.65 million tons in Indonesia in 2018 (10). However, the use of rice bran in Indonesia is generally still limited to animal feed (11).

Several bioactive compounds in rice bran had the potency to inhibit colon cancer cells, that were specifically reported, namely  $\gamma$ -tocotrienol (12),  $\gamma$ -oryzanol (13), and ferulic acid (14). Inhibition of cancer cell proliferation by utilizing the potential of rice bran bioactive compounds is preventive prevention (15), while curative prevention is a type of treatment that has long been applied, such as surgery, radiotherapy, and chemotherapy. Chemotherapy treatment is often reported to cause effects on other organs (16). The chemotherapy agents that have been commonly used, in patients with colon cancer are leucovorin, capecitabine, irinotecan, oxaliplatin, and 5-fluorouracil (17).

Colon cancer itself was ranked the second highest cause of death in the world in 2018 (18). While in Indonesia, ranked the third highest (equivalent to the percentage of people with lung cancer), with the number of incidents in 2018 of 15.245 people, and will continue to increase until 2040 with an estimated number of 27.354 people, based on the 2018 Global Cancer Data (19).

This disease is caused by two main factors, namely internal factors (5-10%) including genetic factors, and external factors (90-95%) such as stress, obesity, radiation, and bad dietary as the biggest contributing factor that is 30% to 35% (20). The effort of preventing cancer can be done, among others, by consuming functional food products. Rice bran which content the bioactive compound can be used as ingredient for development of functional food products.

This article will review the potential of bioactive compounds in rice bran as the prevention of colon cancer. Scientific studies of bioactive compounds and their mechanisms for colon cancer will be reviewed in this article, which will be shown in the form of mapping. Furthermore, the development of rice bran as functional food will also be discussed to provide an illustration of the extent rice bran has been applied as a functional food.

## 2. Bioactive compounds in rice bran and its function

Even though rice bran is a by product of the rice milling process, it contains many essential nutrients such as vitamins, minerals, amino acids, antioxidants (21), bioactive compounds, fats (22), and dietary fiber such as  $\beta$ -glucan, pectin, and gum (23). Fatty acids are dominated by linoleic (31-33%), oleic (37-42%), palmitic (21-26%), and also high in content of polyunsaturated fatty acids, which are known to be good for health (24).

Antioxidants have been reported that has a role in protecting cell damage due to oxidative stress resulting from the formation of free radicals, the oxidative stress is the main cause of cancer cases (25). Groups that act as antioxidant compounds are phenolic acids, anthocyanins, flavonoids, tocotrienols, tocopherols,  $\gamma$ -oryzanol, and phytic acid (26). These groups are found in rice bran. The amount of bioactive compound and nutrient content in rice bran can be seen in Table 1.

Pigmented rice bran has been reported that is rich content in anthocyanin and proanthocyanidin. Both of them have a contribution to pigmented of rice, antioxidant (27,28), anti-inflammatory (29,30), and anthocyanin also act as cytotoxic activity (31). Abdel-Aal *et al* (32) reported, the anthocyanin in black rice bran contained 3.276 mg/g and red rice bran contained 0.094 mg/g. While Hosoda *et al* (33) reported, that anthocyanin was only detected in black rice with the highest concentration, namely the Minenomurasaki cultivar (5.045,6  $\mu$ g/g), while red rice was dominated by the proanthocyanidin component in the Yuyakemochi cultivar (3.060,6  $\mu$ g/g). The variation in the amount of anthocyanin content is due to differences in rice cultivars and location of growth (22).

The compound of  $\beta$ -carotene and lycopene have been reported that it very contributes to the reddish-brown appearance, and both of them are precursors of vitamin A, which can act as antioxidants in the biological system (34).  $\beta$ -carotene and lycopene are part of the carotenoids. These carotenoids are able to bind singlet oxygen and to trap free peroxy radicals, and it is called photoprotective agents (35). Brown rice bran was reported that contains dietary fiber which was four times higher than the white rice (36,27), contained essential amino acids such as lysine (27), and rich in content of vitamins, such as niacin (3.5-5.3), riboflavin (0.04-0.14), thiamine (0.29-0.61), and tocopherol (0.90 –2.50), units of measurement were shown here as mg/100 g of flour (35).

The bioactive component of  $\gamma$ -oryzanol which is present in rice bran (black, red, brown) was reported that had an antioxidant activity of 10 times higher than tocopherol, while tocotrienol had antioxidant activity 40-60 times higher than tocopherol activity (22). These are detected much more in black rice bran. However, all rice bran types contain 4-hydroxy-3-methoxycinnamic acid, which is known to have antioxidative effects and photoprotective (37).

Table 1. Groups of bioactive compounds in rice bran

Bioactive compound	Rice Bran			Reference
	Black	Red	Brown	
Phenolic acids				
Protocatechuic acid (mg/100g)	6.18	5.31	2.87	(38)
p-coumaric acid (mg/100g)	33.35	24.53	16.71	(38)
Ferulic acid (mg/100g)	28.04	23.83	17.79	(38)
Cinnamic acid (mg/100g)	25.53	15.33	9.61	(38)
Syringic acid (mg/100g)	24.40	21.50	14.42	(38)
Sinapic acid (μg/g)	252.10	209.80	258.7	(39)
Gallic acid (μg/g)	161.10	39.00	25.10	(39)
Hidroxybenzoic acid (μg/g)	443.30	52.50	68.90	(39)
Vanillic acid (mg/100g)	36.930	13.83	0.98	(40)
Isoferulic acid (mg/100g)	7.340	8.39	12.34	(41)
Caffeic acid (μg/g)	16.900	24.20	-	(42)
Flavonoids				
Apigenin (mg/100g)	15.31	6.39	4.22	(38)
Luteolin (mg/100g)	10.72	7.74	2.35	(38)
Catechin (mg/100g)	22.05	15.90	8.96	(38)
Myrecitin (mg/100g)	12.85	12.82	5.68	(38)
Quercetin (mg/100g)	15.55	9.27	2.87	(38)
Tricin (μg/g)	10.00	2.40	2.00	(43)
Rutin (μg/g)	2.80	4.10	-	(42)
Isorhamnetin (μg/g)	0.83	-	ND	(44)
Anthocyanins				
Cyanidin-3-glucoside (μg/g)	2316.7	179.0	ND	(39)
Peonidin-3-glucoside (μg/g)	245.7	9.10	ND	(39)
Cyanidin-3-rutinoside (μg/g)	0.70	ND	-	(45)
Steroidal compounds				
γ-oryzanol(mg/g)	9.12	8.58	1.52	(46)
α-tocopherol(μg/g)	43.57	44.00	41.36	(46)
γ-tocopherol(μg/g)	35.31	25.00	37.97	(46)
δ- tocopherol(μg/g)	4.28	4.30	0.25	(45,47)
α-tocotrienol(μg/g)	9.99	11.49	4.36	(45,47)
γ-tocotrienol(μg/g)	53.09	45.83	32.27	(45,47)
δ- tocotrienol(μg/g)	6.03	5.66	2.50	(45,47)
Others				
Protein	13.27	12.93	12.07	(46)
Fat	15.85	17.32	16.96	(46)
Fiber	12.68	12.11	11.77	(46)
Phytic acid	35.00	39.91	48.12	(46)

Information : ND = Not Detected

### 3. The mechanism of bioactive compounds in rice bran as a colon cancer prevention

The prevention mechanism of colon cancer cells by bioactive compounds in rice bran is reported very diverse, starting from acting as an antioxidant so that it can protect against free radicals, changing the cell cycle, cell antiproliferation, modulating the immune system, inducing apoptosis in the caspase cascade pathway, and protecting the layers mucosa by influencing microbial transformation through high fiber content in rice bran (48).

These mechanism were a<sup>9</sup> known different, both of the same or different groups of bioactive compounds, such as ferulic and p-coumaric acid, even though both were phenolic compound group, and capable to delay development in the Caco-2 colon cancer cell cycle, but through a different inhibitory pathway. Ferulic acid delayed on the S phase pathway, aff<sup>4</sup>ted the centrosome central regulatory genes, and DNA damage checkpoint genes such as CEP2, CETN3, and RABGAP1. While p-coumaric acid induced the G2/M phase pathway and affected other cell cycle regulating genes, such as MYC, CDKN1A, PCNA, CDC25A, ODC1, CCNA2, and CCNB1 (49).

Bioactive compound of p-coumaric acid not only played a role in delaying the cell cycle, but it was also reported to have the inhibitory ability on other mechanisms. Supplementation of p-coumaric acid on albino male rats, which was given procarcinogens 1,2 dimethylhydrazine (DMH) could inhibit glucose-regulated protein (GRP78) which was an indicator of transformation into malignant cancer, besides that, p-coumaric acid was able to mediate apoptosis against unfolded protein response (UPR) activated, which was the key to the development of oncogenic by inhibiting the expression of p-p65 (NF- $\kappa$ B) and p-I $\kappa$ B $\alpha$ , and reduced inflammation characterized by the decreased cytokine expression, namely COX-2, IL-6, TNF- $\alpha$  and PGE2 (50).

UPR activation was reported to be able to acti<sup>16</sup>ate anti-apoptotic NF- $\kappa$ B, thus inhibiting apoptotic signals from p53 and inducing angiogenic activity through increased vascular endothelial growth factor (VEGF) (51). The increase of VEGF would cause cancer cells to receive nutrient and oxygen supply so that it was pushed to grow faster, inhibition of VEGF was also known to be regulated by COX-2, 5-LOX (13) and GRP78 enzymes through VEGFR-2 mediating signals (52).

Another component that is also reported to play a role in inhibiting colon cancer is  $\gamma$ -oryzanol. Giving  $\gamma$ -oryzanol as feed to Balb/c mouse transplanted by colon cancer cells CT-26, was able to modulate the im<sup>3</sup>mune system by improving the function of phagocytosis in macrophages, released pro-inflammatory cytokines, tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , and IL-6 by macrophages, increased the activity of natural killer cells (NK), reduced the number of blood vessels in cancer, suppressed vascular endothelial growth factor (VEGF) which was a marker of angiogenesis, and suppressed the COX-2 and 5-LOX enzymes (13). Phagocytosis is very important for cells to protect hosts against harmful foreign particles by swallowing and destroying them, and this process is very important as a form of immune response (53).

Other mechanisms of colon cancer cell inhibition are also reported, namely through the caspase cascade apoptosis pathway. This pathway can kill cancer cells without inflammation and damage to surrounding cells, by mediating by caspase



which will produce an active signaling molecule, which acts as the main link in the regulatory network within the cell, so as to control cell death and inflammation (54). Apigenin (flavonoid group) was reported to be able to increase caspase-8 expression (initiator caspase), and caspase-3 (caspase executor) in HT-29 colon cancer cells, and could reduce cyclin D1 and rapamycin expression. Cyclin D1 acted as a protein that regulated cell cycles, while rapamycin was used as a clinical pathological parameter in colorectal cancer patients (55).

The study of the potential of rice bran as an antiproliferation of colon cancer cells through the mechanism of bioactive compounds, can be seen more comprehensively from the results of *in vitro* and *in vivo* studies presented in Table 2. *In vivo* study studies are presented to strengthen the evidence that the bioactive component present in bran, also works effectively in inhibiting colon cancer cells in experimental animals.

Bioactive compound	Cell/Animal model	Mechanism	Reference
Ferulic acid	Caco-2	Delays the development of the cancer cell cycle especially in the S phase	(49)
	HT29-D4	Inhibits the proliferation of cancer cells, inhibits the production of anion superoxide ( $O_2^-$ ), decreases the cell adhesion, and movement of cancer cells	(56)
	F344 Rats	Reduces the formation of ACF (Aberrant Crypt Foci), reduces the incidence of colon tumors, and increases the activity of quinone reductase (detoxification enzymes)	(57)
p-coumaric acid	Caco-2	Delays the development of the cancer cell cycle especially in the G2/M phase	(49)
	HT29-D4	Inhibits the production of superoxide anion ( $O_2^-$ ) and proliferation of cancer cells, decreases the cell adhesion, and movement of cancer cells	(56)
	HT29; HCT15	Damages the lipid layer and mitochondrial membrane of cancer cells, and increases the oxygen production of reactive species (cancer cells are shrinking)	(58)
	Wistar Rats	Inhibits the preneoplastic lesion, protects the colon from free radicals by acting as antioxidants and detoxifying.	(59)
	Albino Wistar Rats	Induces the apoptosis by decreasing the expression of cytokines COX-2, IL-6, TNF- $\alpha$ , PGE2, p-p65 and p-I $\kappa$ B $\alpha$ , as well as inhibits GRP78 (Glucose Regulated Protein), and mediates apoptosis against active UPR (Unfolded Protein Response).	(50)
Caffeic acid	HT29-D4	Inhibits cancer cell adhesion, cell movement, superoxide anion ( $O_2^-$ ) production, and proliferation	(56)



Gallic acid	HCT15	Damages the lipid layer and mitochondrial membrane in cancer cells, increases the oxygen production of reactive species, and induces apoptosis	(60)
	Albino Wistar Rats	Suppresses oxidative stress, significantly reduces lipid peroxide, and significantly increases the concentration of enzymatic and non-enzymatic antioxidants	(61)
Protocatechuic acid	Colo320; SW480; Caco-2	Induces apoptosis, decreases cancer cell viability, and inhibits DNA synthesis	(62)
Sinapic acid	HT29; SW480	Increases the reactive production of oxygen species and lipid peroxides, damages the mitochondrial membrane in cancer cells, and induces apoptosis.	(63)
<sup>18</sup> Caffeic acid phenethyl ester; caffeic acid phenylpropyl ester	Xenograft Model Mouse	Reduce the number of tumors; reduce PCNA, FASN, and MMP-9	(64)
Tricin	HCA7	Inhibits the activity of the COX-1 and COX-2 cyclooxygenase enzymes (proliferation enzymes), reduces the production of prostaglandin E2 (PGE2)	(65)
	APC <sup>MIN</sup> Mouse	Reduces the number of tumors, and reduces the amount of prostaglandin E2 (PGE2)	(65)
Luteolin	Caco-2	Protects DNA from oxidative damage, and improves activity in cancer cells.	(66)
	SW480; Caco-2	Induces the cell cycle to delay in the G2/M phase	(67)
	Balb/c Rats	Acts as an antimetastatic agent by suppressing the production of MMP-9 and MPP-2	(68)
		Reducing lysosomal enzyme activity, inducing apoptosis by modulating Bcl2, Bax and Caspase-3	(69)
		Reduces MDF (Mucin Depleted Foci) and glycoconjugates levels	(70)

Luteolin	Balb/c Mouse	Reduces the <b>13</b> number of tumors, controls the level of polyamines, and controls the proliferation of cancer cells through inhibition of wnt/ $\beta$ -catenin and GSK-3 $\beta$ pathways. <b>2</b>	(71)
Isorhamnetin	HT29; HCT116; SW480	Delays the cell cycle in the G <sub>2</sub> /M phase, inhibits the PI3K-Akt-mTOR (proliferation) pathway, decreases the protein phosphorylation of Akt (ser473), phospho-p70S6 kinase, and phospho-4E-BP1 (t37/46), and increases the expression of Cyclin B1 protein.	(72)
Apigenin	SW480; HCT15	Inhibits the pathway signal of Wnt/ $\beta$ catenin, thereby suppressing cell proliferation, migration, invasion, and tumor organoid growth.	(73)
	HT-29	Increases the expression of mRNA and caspase-3 and caspase-8 proteins, and decreases the expression of rapamycin (mTOR) and cyclin D1 (CCND1).	(55)
Myrecitin	HCT115	Increases the expression of BAX/BCL-2 ratio, and BAK, and also releases AIF from the mitochondria into the cytosol.	(74)
Rutin	Nude Mice (SW480 Cell Injection)	Reduces VEGF production in rat serum that contains cancer.	(75)
$\gamma$ -tocopherol	HT29	Reduces the potential in the mitochondrial membrane in cancer cells, resulting in the release of cytochrome c compounds that cause activation of caspase 3 (apoptosis)	(76)
	F344 Rats	Reduces the amount of ACF (Aberrant Crypt Foci), decreases the amount of 4-hydroxynonenal, nitrotyrosine, and expression of cyclin D1 in the colon, decreases prostaglandin E2 and 8-isoprostane in serum.	(77)
$\gamma$ -tocotrienol	HCT116; HT29; Caco-2	Suppresses cIAP-1, cIAP-2, survivin (tumorigenk protein) expressions; inhibits the expression of cyclin D1, c-Myc (cell proliferation protein) on HCT116 cells, inhibits expression of MMP-9, VEGF, ICAM-1, CXCR4 (tumor cell invasion protein, metastasis, and angiogenesis), and inhibits NF-kB activation (regulates antiapoptotic protein) in HCT116 cells	(78)

$\gamma$ -tocotrienol	SW620;HCT8	Suppresses protein expression and Wnt/ $\beta$ -catenin signal, cyclin D1, and c-jun	(79)
	HT29	Suppresses the $\beta$ -catenin/Tcf signal (by suppressing the expression of c-myc, cyclinD1 and survivin target genes), thereby inhibits growth and induces apoptosis <sup>8</sup>	(12)
	Xenograft Model Nude Mouse (HCT-116 Transplantation Cell)	Inhibits tumor growth, and decreases the expression of Ki-67, cyclin D1, MMP-9, CXCR4, NF-kB/p65, and VEGF <sup>1</sup>	(78)
$\delta$ -tocopherol	F344 Rats	Reduces the amount of ACF (Aberrant Crypt Foci), decreases the amount of 4-hydroxynonenal, nitrotyrosine, and expression of cyclin D1 in the colon, decreases prostaglandin E2 and 8-isoprostane in serum.	(77)
$\gamma$ -oryzanol	Balb/c Mouse (CT-26 cell transplantation)	Activates natural killer cells, activates macrophages, and inhibits angiogenesis.	(13)
$\beta$ -sitosterol	COLO 320 DM <sup>1</sup>	Increases DNA fragmentation and reactive oxygen production of species, suppresses expression of $\beta$ -catenin and PCNA (marker of cell proliferation)	(80)
Phytic acid	Wistar Rats	Reduces the amount of ACF (Aberrant Crypt Foci) <sup>1</sup> and CM (crypt multiplicity), acts as an antioxidant, and suppresses the expression of $\beta$ -catenin and PCNA <sup>1</sup>	(80)
	Sprague-Dawley Rats	Reduces the amount of ACF (Aberrant Crypt Foci)	(81)
Hemicellulose	F344 Rats	Decreases $\beta$ -catenin expression and COX-2	(82)
		Reduces the number of tumors <sup>7</sup>	(83)

Table 2. Mechanism of inhibition of colon cancer cell proliferation by bioactive compounds in rice bran

#### 4. The development of rice bran as functional food

The development of functional food from rice bran in Indonesia is still very limited. Even though data collection of BPS-Statistics Indonesia (10), Rice production in 2018 was 56.54 million tons, which meant the availability of rice bran potential could reach 5.65 million tons (10), that matter make of the processing of rice bran into functional food, that will have a high economic value. Furthermore, the potential of health is also very promising because the content of bioactive compounds is varied such as high phenolic acids content in nonpigmented rice (1.96 mg GAE/g), red rice bran (4.39 mg GAE/g), and black rice bran 6.65 (mg GAE/g), data were shown here as % dry weight (46), and also contain other bioactive compound such as  $\gamma$ -oryzanol, tocopherol, tocotrienol, anthocyanins, and flavonoids.

Some countries in the world such as the United States, Australia, and Japan have developed rice bran processed products to the commercial stage, such as rice bran cereal, rice bran dessert or energy drinks, rice bran tortillas, rice bran flakes, and rice bran oil. This situation is very different in Indonesia, which are generally still found are traditional foods, such as rice bran bangket, rice bran jenang or rice bran porridge (84). Lack of public awareness about the benefits of rice bran, rice bran quality that has not been standardized, and the lack of downstream industries interested in developing rice bran, become obstacles in the effort to develop rice bran as a functional food (11).

There were some processed rice bran products that had actually been developed at a laboratory scale, such as tempe enriched by rice bran, so resulting in a total phenolic increased by 67% with a ratio of rice bran and soybean (4 :6) (85). Chips products with the main ingredient of wheat flour mixed with bran-enriched tempe had increased protein content by 73% with a ratio of tempe flour and wheat flour (3: 7) (86).

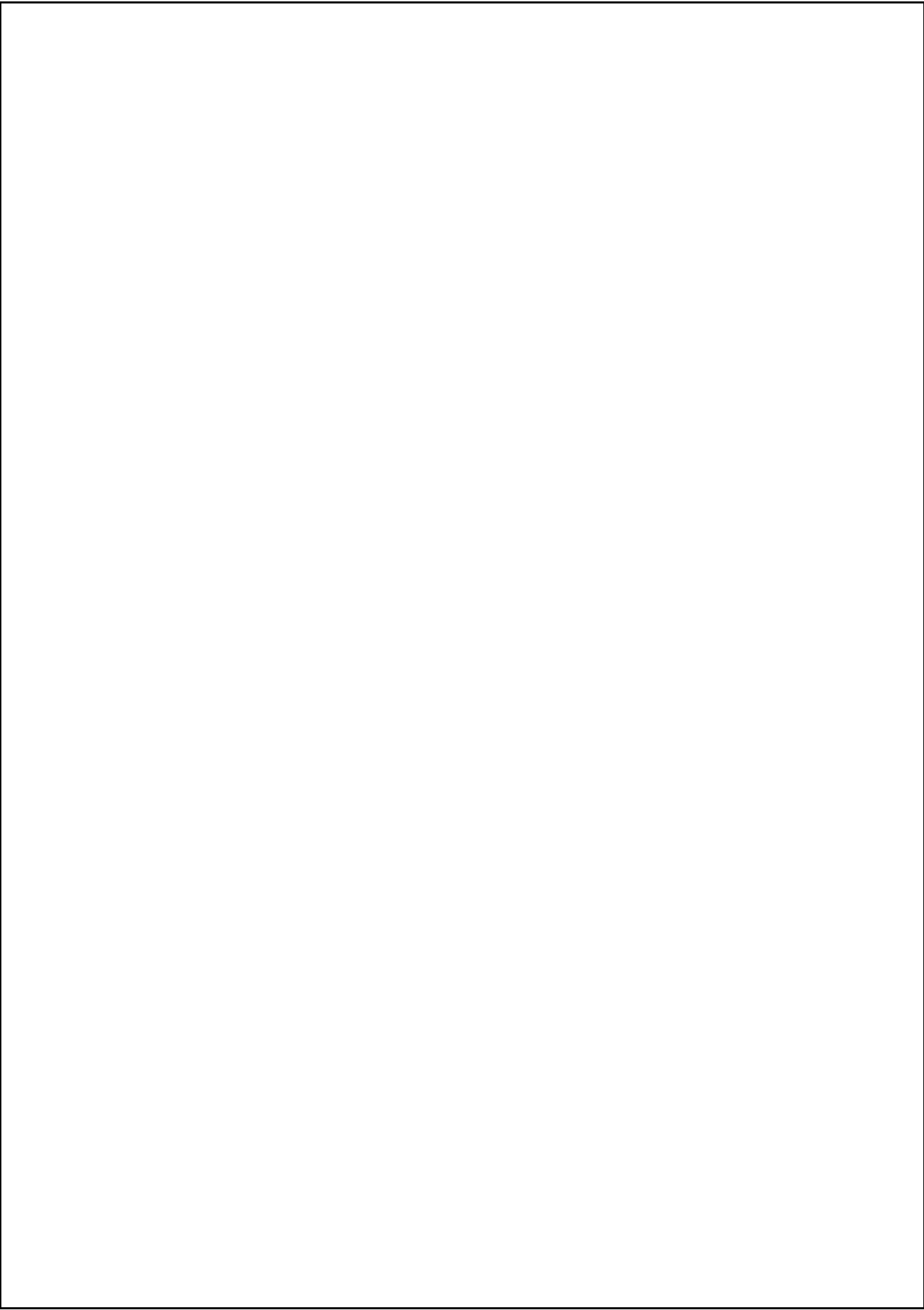
Rice bran cereal (*rice bran puffed cereal*) with the application of twin screw extrusion technology, could produce a crisp texture and crisp resistance time in milk almost the same as or longer than commercial breakfast cereal products (87). Food bar from a mixture of rice bran flour and corn flour (10:90), was able to replace food bars made from wheat flour with insignificant differences in nutritional quality (protein, fat, carbohydrates), and qualify as emergency food with a total energy of 232.43 kcal/50 g of the ingredient (88). Furthermore, extrusion products from a mixture of rice and rice bran were reported to contain sufficient nutritional value and had the potential to be developed into snack products (89).

The introduction of rice bran as a functional food is important to do. One way is by highlighting its health benefits as a marketing strategy. Thus, it is hoped to open the community paradigm and increase interest in the downstream industry as an effort to develop functional food from rice bran.

#### 5. Conclusion

The bioactive compounds in rice bran consist of several categories, such as phenolic acids, flavonoids, anthocyanins, and steroidal compounds. The mechanisms of the bioactive compound rice bran in preventing colon cancer was classified by its function as an antioxidant, damage of the lipid layer and

mitochondrial membrane, activation of immune cells, modulation of the cell cycle, inhibition of protein invasion of tumor cells, metastases, and angiogenesis, and activation of protein caspase to encourage apoptosis. The development of rice bran itself as a functional food product in Indonesia, is still on a laboratory scale, although some are developed into traditional foods. Educating the public about the benefits of rice bran for health is a strategy for product development from rice bran raw material in the future.





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