Physicochemical, Antioxidant and Sensory Properties of Wheat Flour Cracker Substituted with Riceberry Flour

คุณสมบัติทางเคมีกายภาพ ความสามารถในการต้านอนุมูลอิสระ และคุณสมบัติทางประสาทสัมผัสของแครกเกอร์ที่ทดแทนแป้งสาลีด้วยแป้งข้าวไรซ์เบอร์รี่

Napassorn Payantakom (นภัสสร พยรรตาคม)* Porntip Pasukamonset (พรทิพย์ พสุกมลเศรษฐ์)**
Kittana Mäkynen (กิตณา แมคีเน็น)*** Sirichai Adisakwattana (สิริชัย อดิศักดิ์วัฒนา)****
Winai Dahlan (วินัย ดะห์ลัน)***** Sathaporn Ngamukote (สถาพร งามอุโฆษ)******

ABSTRACT

This study aimed to investigate the effect of partial substitution of wheat flour with riceberry flour (0, 5, 10, 15 and 20% of wheat flour weight) on physicochemical, antioxidant and sensory properties of crackers. Our results indicated that redness (a*) and hardness values of crackers significantly increased, while the lightness (L*) and yellowness (b*) values significantly decreased with an increase of riceberry flour levels ($P < 0.05$). In addition, total polyphenols, anthocyanins and antioxidant property significantly increased in crackers incorporated with riceberry flour ($P < 0.05$). Surprisingly, the substitution of wheat flour with 20% riceberry flour showed the highest score of flavor, taste and overall acceptability when compared to control. Thus, the replacement of wheat flour with 20% riceberry flour can enhance antioxidant property without affecting consumer acceptance of the food products.

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการทดแทนแป้งสาลีด้วยแป้งข้าวไรซ์เบอร์รี่ร้อยละ 0, 5, 10, 15 และ 20 (ของน้ำหนักแป้งสาลี) ในผลิตภัณฑ์แครกเกอร์ต่อคุณภาพทางเคมีกายภาพ ความสามารถในการต้านอนุมูลอิสระ และ คุณภาพทางประสาทสัมผัส ผลการทดลองพบว่า ค่าสีแดง (a*) และค่าความแข็งของแครกเกอร์เพิ่มขึ้น ในขณะที่ค่าความสว่าง (L*) ค่าสีเหลือง (b*) ลดลงอย่างมีนัยสำคัญทางสถิติ เมื่อระดับการทดแทนแป้งข้าวไรซ์เบอร์รี่เพิ่มขึ้นในแครกเกอร์ ($P < 0.05$) นอกจากนี้ยังพบว่าปริมาณโพลีฟีนอลิกทั้งหมด ปริมาณแอนโทไซยานิน และประสิทธิภาพการต้านอนุมูลอิสระเพิ่มขึ้นผลิตภัณฑ์แครกเกอร์ที่มีส่วนผสมของแป้งข้าวไรซ์เบอร์รี่ ($P < 0.05$) เป็นที่น่าสนใจอย่างยิ่งเมื่อทดสอบความชอบของผลิตภัณฑ์พบว่า แครกเกอร์ที่ทดแทนแป้งสาลีด้วยแป้งข้าวไรซ์เบอร์รี่ร้อยละ 20 ได้รับคะแนนความชอบสูงสุดในด้าน กลิ่นรส รสชาติ และความชอบโดยรวมเมื่อเปรียบเทียบกับสูตรควบคุม ดังนั้น การทดแทนแป้งสาลีด้วยแป้งข้าวไรซ์เบอร์รี่ร้อยละ 20 สามารถเพิ่มประสิทธิภาพการต้านอนุมูลอิสระโดยไม่ส่งผลกระทบต่อการยอมรับทางประสาทสัมผัส

Keywords: Cracker, Riceberry Rice, Antioxidant
คำสำคัญ: แครกเกอร์ ข้าวไรซ์เบอร์รี่ การต้านอนุมูลอิสระ

** Student, Master of Science Program in Food and Nutrition, Faculty of Allied Health Sciences, Chulalongkorn University*
*** Lecturer, Department of Home Economics, Faculty of Agriculture, Kasetsart University*
**** Lecturer, Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University*
***** Associate Professor, Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University*
****** Associate Professor, The Halal Science Center, Chulalongkorn University*
******* Assistant Professor, Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University*

Introduction

Crackers, thin and crisp wafer or biscuits made by unsweetened and unleavened dough are one of the bakery products which are highly consumed by worldwide consumers. However, they are usually high in fat and low nutritive value (Sedej et al., 2011; Shukla, 1994). Since consumers are increasingly interested in healthy and safe products, many food industries try to develop foods with additional health benefits such as anthocyanin-rich food because of its antioxidant properties (Ahmed and Abozed, 2014). Recently, many scientific evidences have illustrated an important role of food with high antioxidants properties in order to prevent degenerative diseases such as cancer cardiovascular diseases and diabetes mellitus. Therefore, there is a lot of interest in incorporation of raw materials from natural plants and fruits such as blueberries (Scibisz et al., 2012), black rice (Mau et al., 2016), purple corn (Guo et al., 2018), purple sweet potato (Li et al., 2017), and cherry (Casedas et al., 2018) into the products due to their high in phytochemical compounds, especially anthocyanin resulting in improving the quality of food products.

In Thailand, Rice (*Oryza Stiva* L.), is the most important staple food as a daily meal which provides health benefits and contains several nutrients. Riceberry rice, black purple rice variety, a cross-bred between the Khao Hom Nin Rice and Khao Hom Mali 105 which is a newly registered rice variety from Thailand developed by the Rice Science Center, Kasetsart University (Sirichokworrakit et al., 2015). It has high anthocyanin content especially cyanidin-3-glucoside and peonidin-3-glucoside which have strong antioxidant effects (Yodmanee et al., 2011). Nowadays, riceberry rice is one of the most popular rice consumed among consumers because it has shown health-promoting properties including attenuating a rise of blood glucose (Kongkachuichai et al., 2013), anti-inflammation (Adriano et al., 2006) and anti-aging (Chiang et al., 2006). In addition, riceberry rice has been used as a raw material to increase nutrient density in many food products. Moreover, recent published works have demonstrated the effect of partial substitution of wheat flour with riceberry flour on quality of food products including noodles (Sirichokworrakit et al., 2015) which could improve the quality and also provided a source of colorant in the products. Furthermore, the supplementation of riceberry flour into low-fat ice-cream (Chuaykarn et al., 2013) and salad dressing (Tantivirasut et al., 2014) showed the increase of total phenolic content, total anthocyanin content and antioxidant activities. However, no study demonstrates the development of high nutritive value and functionality of riceberry rice crackers. Therefore, the new riceberry cracker would be good functional food choices which present a good source of antioxidants.

Objectives of the study

The aim of this study was to investigate the effect of partial substitution of wheat flour with riceberry flour on physicochemical, antioxidant properties and sensory evaluation of crackers.

Materials and Methods

Materials

All ingredients used for the crackers were purchased from a supermarket in Bangkok, Thailand including, riceberry flour (Sunfood Corp Co., Ltd., Samutprakan, Thailand), an all-purpose flour (United Flour Mill Public Co., Ltd., Samutprakan, Thailand), salt (Thai Refined Salt Co., Ltd., Nakhonratchasima, Thailand), sugar (Mitr Phol Sugar Corp Co., Ltd., Suphanburi, Thailand), yeast (Greathill Co., Ltd., Bangkok, Thailand), butter (KCG Corp Co., Ltd., Bangkok, Thailand) and shortening

(Thai Vegetable Oil Public Co., Ltd., Bangkok, Thailand). The riceberry flour was passed through a 100-mesh screen and was sealed in an aluminum bag and kept at room temperature before use. Methanol, hydrochloric acid, Folin-Ciocalteau reagent, sodium carbonate, gallic acid were purchased from Fluka® Analytical (St. Louis, MO, USA). Potassium chloride, Sodium acetate, Ascorbic acid and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Preparation of crackers

The basic crackers formula consisted of 54.4 g of all-purpose flour, 0.3 g of salt, 0.6 g of yeast, 13 g of butter, 4.3 g of shortening, 1 g of sugar, and 26.1 g of water. Riceberry crackers were prepared by substitution of wheat flour with 5%, 10%, 15% and 20% riceberry flour. All ingredients were blended and mixed to form a cohesive dough. The dough was wrapped with plastic wrap for 10 min at room temperature. Then, the dough was rolled into 3 mm thickness and cut by pressing rectangle mold (40 mm diameter) into pieces. The crackers were baked at 180 °C for 10 min. Baked crackers were cooled at ambient temperature. Finally, the crackers were packed in an aluminum bag until physicochemical, phytochemicals, antioxidants properties and sensory evaluations were analyzed (Klunklin and Savage, 2018).

Physicochemical properties of crackers

Texture analysis

The texture analysis of the crackers was measured by a texture analyzer TA.XT. Plus (UK). The hardness and fracturability of the crackers were measured using a spherical stainless probe, 5 mm thick. The following settings was used: pre-test speed 5.0 mm s^{-1}, test speed 1.0 mm s^{-1}, post-test speed 5.0 mm s^{-1}, distance 7.0 mm. Fifteen replicates of crackers were determined (Sattasuwan et al., 2010).

Color analysis

The lightness (L*), redness (a*) and yellowness (b*) values of the crackers were determined by using Hunter Lab colorimeter (Model: ColorFlex 4.5/0, USA). The instrument was calibrated with black and white standard tiles before color measurement. Five replicates of crackers were determined (Ahmed and Abozed, 2014).

Samples extraction

Samples (5 g) were extracted with 25 ml of methanol containing 15% HCI in shaking water bath at 25 °C for 24 h. The extracted were filtered using filter paper (Whatman No. 1) and were then centrifuged at 3000 rpm for 15 min. The supernatant was removed and dried using a rotary evaporator at 50 °C. The crude extract was kept at -20 °C until the phytochemicals and antioxidant capacity analysis (Shen et al., 2009).

Determination of total phenolic content

The total phenolic content (TPC) was determined by the Folin-Ciocalteu assay. Briefly, the extracted samples (20 µl) were mixed with 150 µl of the freshly diluted 10-times Folin-Ciocalteu reagent. After incubation at room temperature for 5 min, 150 µl of sodium carbonate (6% w/v) were added to the mixture and incubated at room temperature for 1 h. The absorbance at 760 nm was measured using a spectrophotometer (BioTek instruments, USA). Gallic acid (0-0.2 mg/ml) was used as a standard (Shen et al., 2009).

Determination of total anthocyanins content

The total anthocyanins contents (TAC) were determined using pH colorimetric method. The extracts were measured at 510 and 700 nm in pH 1.0 of 0.025 M potassium chloride (KCl) buffer and in pH 4.5 of 0.4 M sodium acetate (CH_3COONa) buffer. The sample (600 µl) was mixed with 600 µl of each buffer and incubated for 20 min at room temperature. The absorbance (Abs) was calculated using an equation of $A = (Abs_{510} – Abs_{700})_{pH1.0} - (Abs_{510} – Abs_{700})_{pH4.5}$, MW = molecular weight of cyanidin-3-glucoside = 449.2 g/mol, DF = dilution factor, l = path length in cm, ε = 26,900 molar extinction coefficient, in L/mol/cm for cyanidin-3-glucoside, 10^3 = factor for conversion from g to mg (Lee et al., 2005).

Determination of DPPH radical-scavenging

The extracts (100 µl) were mixed with 100 µl of a DPPH solution (0.2 mM in methanol) and incubated for 30 min at room temperature. The absorbance was measured at 517 nm and extract concentration providing 50% inhibition (IC_{50} value) was calculated from the plotted graph of percentage inhibition against the concentrations of the extracts. Ascorbic acid was used as standard curve which was prepared within the range of 0-50 µg/ml (Jariyapamornkoon et al., 2013).

Sensory evaluation

The untrained panelists (n = 50) were recruited from Chulalongkorn University. A 9-point hedonic scale was used to evaluate all attributes including appearance, color, flavor, taste, hardness, and overall acceptance. The samples were presented to the panelists in a random order with a white plate labeled with three-digit random codes. Attributes were scored on a scale varying from “9 = like extremely” to “1 = dislike extremely”. Drinking water were provided to the panelists to rinse their mouth between the samples (Mir et al., 2015).

Statistical analysis

Data were reported as mean of values ± SEM. Statistical analysis were performed by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test using SPSS 17.0 (SPSS Inc., Chicago, USA) The significance different test was used for mean comparison and P-value < 0.05 was considered statistically significant.

Results

The physical characteristics (texture and color properties) of crackers substituted with riceberry flour were presented in Table 1. The incorporation of riceberry flour affected the hardness of riceberry cracker by increasing its value from 4112.98 ± 14.10 to 5354.81 ± 65.93 g when compared to control. In addition, the results of the hunter L*, a* and b* values corresponding to lightness, redness, and yellowness, respectively showed variation depended on the incorporation of riceberry flour level. As expected, the replacement of wheat flour with riceberry flour affected the color of the crackers. The incorporation of riceberry flour significantly reduced L* and b* values in the crackers with increased riceberry flour level ($P < 0.05$). In contrast, a* value of the cracker was significantly increased when the replacement of riceberry flour with wheat flour level increase ($P < 0.05$).

As shown in Table 2, riceberry flour contained total phenolic content (525.85 ± 11.18 mg GAE/100 g flour) and total anthocyanins (52.57 ± 3.87 mg Cy-3-glu /100 g flour). Moreover, riceberry flour also demonstrated antioxidant properties via scavenging DPPH, a stable free radical, with IC_{50} value of 0.22 ± 0.01 mg/ml. It was found that the incorporation of riceberry flour into crackers significantly increased total phenolic and total anthocyanins contents of riceberry crackers with the increased level of riceberry flour compared to control crackers (Table 3). Total phenolic and total anthocyanins contents of cracker significantly increased from 2.33 ± 0.28 (control) to 8.94 ± 0.14 mg GAE/10g cracker (20%RBF) and from 31.26 ± 0.28 (control) to 803.92 ± 5.29 µg Cy-3-glu /10g cracker (20%RBF), respectively (Table 3). In addition, the substitution of wheat flour with riceberry flour considerably enhanced antioxidant properties in the cracker with the increased level of substitution of riceberry flour. The IC_{50} value of DPPH radical scavenging ability in the crackers significantly reduced from 0.69 ± 0.01 to 0.41 ± 0.01 mg/ml with the incorporation of riceberry flour increase in the cracker ($P < 0.05$) as demonstrated in Table 3.

Crackers incorporated with different level of riceberry flour were evaluated for their sensory qualities in all attributes including appearance, color, flavor, taste, hardness and overall acceptability as shown in Table 4. The results indicated that no statistically significant differences in appearance and color scores between the control cracker and cracker incorporated with riceberry flour (5-20%) were found but the addition of 20%RBF in the cracker significantly decreased hardness score from 7.32 ± 0.11 (control) to 6.90 ± 0.20 (20%RBF). However, these scores were still in slightly to moderately acceptable level (score 6-7). Surprisingly, the flavor, taste and overall acceptability scores of crackers significantly increased when 20% riceberry flour was added in the crackers ($P < 0.05$) as shown in Table 4.

Table 1 Physical characteristics of cracker substituted with various levels of riceberry flour (RBF).

Samples	Control	5%RBF	10%RBF	15%RBF	20%RBF
Texture					
Hardness (g)	4112.98 ± 14.10^{a}	4238.65 ± 70.64^{ab}	4384.56 ± 85.08^{bc}	4471.74 ± 12.79^{c}	5354.81 ± 65.93^{d}
Color					
L*	40.2 ± 0.21^{a}	22.48 ± 0.22^{b}	17.00 ± 0.24^{c}	15.66 ± 0.15^{d}	12.34± 0.17^{e}
a*	0.22 ± 0.003^{a}	3.15 ± 0.05^{b}	3.68 ± 0.06^{c}	3.72 ± 0.07^{c}	3.97 ± 0.02^{d}
b*	10.31 ± 0.32^{a}	3.16 ± 0.10^{b}	2.39 ± 0.09^{c}	2.00 ± 0.11^{c}	1.28 ± 0.03^{d}

Values are expressed as mean ± SEM (n = 3). Mean with different superscript letter within a row are significantly different ($P < 0.05$).

RBF = Riceberry flour

Table 2 Phytochemical contents and antioxidant properties of riceberry flour.

Sample	Total Phenolic content	Total Anthocyanins	DPPH
	(mg GAE/100 g)	(mg Cy-3-glu /100 g)	IC_{50} (mg/ml)
Riceberry flour	525.85 ± 11.18	52.57 ± 3.87	0.22 ± 0.01

GAE = Gallic Acid Equivalent; Cy-3-glu = Cyanidins-3-glucosides.

Values are expressed as mean ± SEM (n = 3).

Table 3 Phytochemical and antioxidant properties of cracker substituted with various levels of riceberry flour (RBF).

Sample	Total Phenolic	Total Anthocyanins	DPPH
	(mg GAE/10g cracker)	(µg Cy-3-glu /10g cracker)	IC_{50} (mg/ml)
Control	2.33 ± 0.28^{a}	31.26 ± 0.28^{a}	0.69 ± 0.01^{a}
5%RBF	3.30 ± 0.05^{b}	153.45 ± 14.77^{b}	0.62 ± 0.02^{b}
10%RBF	4.17 ± 0.08^{c}	369.48 ± 34.09^{c}	0.57 ± 0.01^{c}
15%RBF	7.44 ± 0.32^{d}	623.90 ± 31.79^{d}	0.44 ± 0.02^{d}
20%RBF	8.94 ± 0.14^{e}	803.92 ± 5.29^{e}	0.41 ± 0.01^{d}

GAE = Gallic Acid Equivalent; Cy-3-glu = Cyanidins-3-glucosides.

Values are expressed as mean ± SEM (n = 3). Mean with different superscript letter within a column are significantly different ($P < 0.05$).

RBF = Riceberry flour

Table 4 Sensory evaluation scores of cracker substituted with various levels of riceberry flour (RBF).

Samples	Appearance	Color	Flavor	Taste	Hardness	Overall acceptability
Control	7.26 ± 0.15^{a}	7.12 ± 0.19^{a}	6.26 ± 0.19^{a}	6.82 ± 0.11^{a}	7.32 ± 0.11^{a}	7.12 ± 0.13^{a}
5%RBF	7.08 ± 0.10^{a}	7.04 ± 0.08^{a}	7.02 ± 0.10^{b}	6.94 ± 0.09^{a}	7.38 ± 0.11^{a}	7.08 ± 0.11^{a}
10%RBF	7.12 ± 0.12^{a}	7.10 ± 0.08^{a}	7.12 ± 0.11^{bc}	7.06 ± 0.14^{ab}	7.16 ± 0.13^{ac}	7.24 ± 0.11^{a}
15%RBF	7.18 ± 0.14^{a}	7.26 ± 0.12^{a}	7.40 ± 0.11^{cd}	7.26 ± 0.14^{b}	7.04 ± 0.19^{b}	7.50 ± 0.13^{b}
20%RBF	7.34 ± 0.14^{a}	7.30 ± 0.14^{a}	7.50 ± 0.09^{d}	7.54 ± 0.10^{c}	6.90 ± 0.20^{bc}	7.70 ± 0.09^{b}

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

RBF = Riceberry flour

Discussion

The incorporation of riceberry flour into the crackers clearly affected texture quality with increasing hardness of cracker fortified with riceberry flour due to the lower gluten content in crackers. Gluten protein helps dough rise by trapping carbon dioxide during dough fermentation (Klunklin and Savage, 2018). Therefore, Riceberry flour, a gluten-free flour, cannot generate a viscoelastic network like gluten protein in wheat flour. In addition, total phenolic and anthocyanin contents containing in the riceberry flour may also cause conformation changes of the protein in the products resulting in decreasing elasticity of crackers (Saikia et al., 2012). These texture characteristic qualities are in agreement with the other previous findings that studied of cracker incorporation with buckwheat flour (Torbica et al., 2012) and waxy rice flour (Giuberti et al., 2017). Recently, Pasukamonset and colleague also demonstrated that the sponge cakes fortified with *Clitoria ternatea* extract (CTE), high anthocyanin containing product, increased hardness when increasing level of the extract in the sponge cakes (Pasukamonset et al., 2018).

Since color and appearance play a crucial role in the purchase process, the L*, a*, and b* color scale is important to determine in cracker incorporated with riceberry cracker (Sozer et al., 2014; Klunklin and Savage, 2018). The incorporation of riceberry flour caused the dark purple color of cracker as expected. This could be due to the anthocyanins in riceberry flour which are water-soluble pigments responsible for red, purple and blue colors in plants, fruit, and vegetables (Khoo et al., 2017). In addition, the oxidation reaction of total phenolic content in rice berry flour during the thermal process also involved in color changes of the crackers (Al-Sayed et al., 2013; Mau et al., 2016). The similar findings in the previous studies showed that the addition of apple pomace (Mir et al., 2015) and *Hibiscus sabdariffa* by-product in the cracker (Ahmed and Abozed, 2014) caused a reduction of L* value together with an increasing of a*, and b* values.

The increasing of all phytochemical compounds (total phenolic and total anthocyanins) and antioxidant activity (DPPH) were found in crackers enriched riceberry flour. These results might be due to the high amount of polyphenolic and anthocyanins contents presented in riceberry flour. The previous study suggested that the purple rice is an excellent source of phenolic compounds, especially anthocyanins as the present of the dark-purple pigment in the rice (Jang, Xu 2009; Loypimai et al., 2016). The major abundant anthocyanins of purple or black rice were cyanidin 3-glucoside (C3G) and peodinin-3-glucoside (P3G) which have been recognized as health-enhancing substances owing to their antioxidant activity (Abdel-Aal et al., 2006; Klunklin and Savage, 2018). The strong effect of antioxidant activities of the crackers enriched riceberry flour may link to the concentration of total phenolic and total anthocyanins. Similar results were also demonstrated in sponge cakes fortified with CTE (Pasukamonset et al., 2018), the addition of mango peel powder by-products into crackers (Ajila et al., 2010) and biscuits supplementation with purple rice flour (Klunklin and Savage, 2018). However, the phytochemical compounds and antioxidant activity in the food products may decrease because the thermal process such as baking method lessen total polyphenolic, flavonoids, and anthocyanins compounds by breakdown or conversion of these active compounds to other products resulting in decreasing their antioxidants properties (Saikia et al., 2012; Sirichokworrakit et al., 2015; Pasukamonset et al., 2018).

Regarding the sensory evaluation, the 9-point hedonic scale is the most often used to test foods, beverages and consumer products because of its simple and effective method (Tuorila, 2015). In the present findings, it was found that sensory evaluation scores related to the changes of physical characteristics of cracker incorporated with riceberry flour. The hardness of the riceberry rice crackers increased with an increase of riceberry flour levels resulted in lower the likeness score of the hardness. The same trend was also found in sponge cake supplemented with CTE (Pasukamonset et al., 2018). The flavor and taste scores of riceberry rice crackers increased when increasing in riceberry flour substitution levels. Similar results were also reported in gluten-free rice flour biscuits which prepared by incorporated brown rice into biscuits (More et al., 2013). The increase of liking scores of the taste in the crackers incorporated with riceberry cracker indicated that riceberry flour has developed the satisfiable flavor of the products. Although the addition of riceberry flour affected the color different of cracker, the liking scores of appearance and color were comparable with control crackers. However, it should be mentioned that the combination of the ingredients together with baking process have an important effect on the quality and color of the final product (Sudha et al., 2007). In this study, the score of overall acceptability significantly increased with the higher level of riceberry flour incorporated in the crackers. Therefore, a partial replacement of wheat flour with 20% riceberry flour in crackers is well accepted by consumer.

Conclusion

The addition of riceberry flour into crackers significantly increased hardness and redness together with a concomitant decrease lightness and yellowness of the food products. Additionally, cracker incorporated with riceberry flour increased total phenolic content and anthocyanins resulted in increasing antioxidant property with an increased level of riceberry flour. Moreover, the replacement of wheat flour with 20% of riceberry flour not only improved flavor of the products but also increased overall appreciation score of the crackers. Therefore, riceberry flour could be used as an ingredient for developing novel high nutritional value crackers with enhancing antioxidant property and well accepted by the consumers.

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