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FULL PAPER

The improvement of nutrition quality, antioxidant capacity, and functional properties of cowpea (*Vigna unguiculata*) sprout flour through NaCl and Na-alginate elicitation

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Abstract

Cowpea exhibits potential antioxidant capacity, and various health benefits, as well as a good source of protein and nutraceutical compounds. Our previous study showed that elicitation using 50 mM NaCl or 250 ppm Na-alginate before germination is the most effective technique to improve the antioxidant capacity of cowpea sprout with the lowest impact on the reduction of germination power and yield. The objective of this research was to evaluate the potential of NaCl and Na-alginate elicitation on the improvement of the nutrition quality (proximate), antioxidant capacity (total phenolic content, total flavonoid content, reducing power, and Trolox equivalent antioxidant capacity (TEAC)), and functional properties (water absorption capacity (WAC), oil absorption capacity (OAC), water holding capacity (WHC), oil holding capacity (OHC) and emulsion stability (ES)) of cowpea sprout flour. The cowpea sprout was prepared with and without elicitation (control) by immersing the cowpea seeds either in 50 mM NaCl, 250 ppm Na-alginate or distillate water for 8 hrs prior to germination for 48 hrs. The sprouts then were dried, milled, and sieved to produce sprout flour. The research results proved that elicitation significantly increases the protein and total mineral levels, functional properties, and antioxidant capacity, and reduces the fat and carbohydrate levels of cowpea sprout flour. Elicitation using Na-alginate produced a higher improvement of OHC, nutrition, and antioxidant capacity of elicited cowpea sprout flour than that of NaCl elicitation. This study provides an alternative strategy for improving the nutrition, antioxidant capacity, and functional properties of legume flour.

1. Introduction

Cowpea (*Vigna unguiculata*) is multifunctional leguminous that has some superiorities, among others a remarkable source of nutraceutical compounds such as dietary fiber, antioxidants, and folic acid, poses high protein content (reach 32% db), low-fat (1% db), high tolerance to environmental stresses as well as the potential to restore soil fertility. When compared to soybeans as the most common beans, cowpea has a lower fat content and higher levels of productivity (Ariviani *et al*., 2020a; Ariviani *et al*., 2020b). Cowpea is reported as a potential antioxidant source due to its phenolic and flavonoid compounds. Cowpea exhibits potential health benefits, such as anti-diabetes and anti-cancer (Ariviani *et al*., 2020b). Sombié *et al.* (2018) reported that the antioxidant capacity and other health-promoting properties of cowpea are related to the phenolic compounds. The major phenolic compounds of cowpea are flavonoids and phenolic acids. Unfortunately, similar to other legumes, cowpea also possesses anti-nutritional compounds such as phytic acid, enzymes inhibitor, and oligosaccharides which decrease the nutritional quality (Jayathilake *et al.*, 2018). Devi *et al.* (2015) reported that germination significantly improves the nutritional quality of cowpea by decreasing trypsin inhibitor activity, increasing protein content, and protein digestibility.

Germination has been known as a simple and effective technique to reduce anti-nutritional compounds and increase protein content, thus improving the nutritional quality of legumes (Xu *et al*., 2019). Additionally, the formation of the antioxidant compounds is also triggered during germination, thus

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leading to increases in the antioxidant activity of legumes (Benincasa *et al*., 2019), as observed in cowpea (Doblado *et al*., 2007).

Germination of cowpea with elicitation technique using abiotic (50 mM NaCl) and biotic (250 ppm Na-alginate) elicitors is the most convincing method for enhancing the total phenolic content and antioxidant activity of cowpea sprout (Ariviani *et al*., 2020a). Germination by elicitation technique using abiotic or biotic elicitors proved to be capable to improve nutritional quality and antioxidant capacities of the legumes sprouts (Benincasa *et al*., 2019), such as in lentils (*Lens culinaris*) (Świeca, 2015), and pigeon pea (*Cajanus cajan*) (Ariviani *et al*., 2020c; Ariviani *et al*., 2021). Legumes sprouts can be processed into flour to extend their shelf life while expanding their application. Recently, the author's study exhibited that elicited cowpea sprout flour with the highest levels of whiteness degree, antioxidant activity, and dissolved protein could be prepared at the drying temperature of 80°C for 2 hrs (Ariviani *et al.,* 2020a).

The research objective was to examine the enhancement of antioxidant capacity, nutritional quality, and functional properties of cowpea sprout flour through elicitation prior to germination, i.e., by submerging in 50 mM NaCl as an abiotic elicitor and 250 ppm Na-alginate as the biotic elicitor. Several studies reported increases in the functional properties of legumes flour due to the germination treatment, such as in pigeon pea (*Cajanus cajan*) (Ariviani *et al*., 2020c), and mung bean (*Vigna radiata*) (Liu *et al*., 2018). Nevertheless, to the best of our knowledge, the potential of elicitation in the functional properties improvement of cowpea sprout flour has not been reported. Flour functional properties show the complex interaction between the composition, molecular conformation, structure, and physicochemical properties of flour with the nature of the environment. It reflects how the flour will interact with other food components directly or indirectly affecting processing applications, food quality, and ultimate acceptance. The flour functional properties determination is important to predict the flour application in suitable food or beverage products (Chandra *et al*., 2015; Ohizua *et al.*, 2017).

2. Materials and methods

The materials used in this study are light brown cowpea (*Vigna unguiculata*), and soybean oil "Mazola" obtained from the local market in Surakarta, Indonesia. Sodium alginate, Folin-ciocalteau's phenol reagent, gallic acid, Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid), quercetin, potassium persulfate, and ABTS (2,2'Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Sodium chloride, sodium carbonate, ascorbic acid, sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dihydrate, trichloroacetic acid, potassium ferricyanide, ferric chloride hexahydrate, aluminum chloride, sodium acetate, ethanol, methanol, and petroleum ether were purchased from Merck Millipore Co. (Darmstadt, Germany). All chemicals used in this study were analytical grade.

2.1 Preparation of cowpea sprout

The cowpea sprout preparation was conducted according to Rajendra *et al.* (2019) with slight modifications. Briefly, cowpea seeds (not wrinkled nor hollow) were submerged in 50 mM NaCl or 250 ppm Na-alginate solution (elicitation treatment) or in distilled water (control) with a ratio of 1:3 w/v for eight hrs and then were germinated for 48 hrs at room temperature. During germination, the seeds were sprayed with distilled water every 12 hrs. The elicitor used in this study refers to Ariviani *et al*. (2020a).

2.2 Preparation of the cowpea sprout flour

The cowpea sprout flour was prepared according to Ariviani *et al*. (2020a). The cowpea sprouts were dried at a temperature of 80°C for 2 hrs in a cabinet dryer (Xingtai XTDQ-101-4, Jiangsu, China). The dried sprouts were milled and further sieved using a 60-mesh sieve (Virsair CF812-01, Indonesia).

2.3 Evaluation of nutrition quality and functional properties of flour

The evaluation of the nutrition quality of flours was performed by Proximate analysis according to AOAC standard methods (AOAC, 1990). The functional properties of the flours were evaluated by measuring the WAC and OAC using the method according to Jan *et al*. (2015), WHC and OHC with the method previously described by Nguyen *et al*. (2015), and ES tested according to the method of Yasumatsu *et al*. (1972).

2.4 Antioxidant capacity determination

The antioxidant capacity determination was started through extract preparation of the cowpea sprout flours. Cowpea sprout flour prepared with and without elicitation was extracted with methanol-water (80:20) at the ratio of (1:10) using a water bath shaker (SWB 20, Fisher Scientific Haake, Germany) at 50°C and 200 rpm for 2 hrs. The extract was centrifuged using PLC-05 Centrifuge (Gemmy, Taiwan) at 10000 rpm for 15 mins. The clear supernatant was collected and stored in amber bottles at 10°C until further analysis. Determination of antioxidant capacity was conducted by measuring the total phenolic content (TPC), total flavonoids content (TFC), reducing power (RP), and Trolox equivalent antioxidant capacity (TEAC). The TPC was evaluated according to the method described by Singleton *et al*. (1999), and it was expressed as mM Gallic acid equivalent per 100 g flour dry weight (mM GAE/100 g db). TFC was determined using the method previously described by Pękal and Pyrzynska (2014) and expressed as mM Quercetin equivalent per 100 g flour dry weight (mM QE/100 g db). The method used for RP analysis was previously described by Berker *et al*. (2007). The RP expressed as mM Ascorbic acid equivalent activity per 100 g flour dry weight (mM AAEA/100 g db). TEAC was analyzed using ABTS•+ free radical method (Re *et al*., 1999) and expressed as mM Trolox equivalent antioxidant capacity per 100 g flour dry weight (mM TEAC/100 g db).

2.5 Statistical analysis

Data analysis was conducted by the IBM SPSS Statistics 25 program (SPSS Inc., Chicago, USA) using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) to evaluate the significant difference between the means ($p < 0.05$). Data were presented as mean±standard deviation (SD) of the three replications.

3. Results and discussion

3.1 The nutrition quality of cowpea sprout flour

Cowpea is known as a nutrient-dense food, but it also contains anti-nutritional compounds that decrease its nutritional quality. Germination and other processing methods proved capable to improve the nutritional quality of cowpea by altering the nutrient contents besides reducing the anti-nutrient compounds (Jayathilake *et al*., 2018). Therefore, it is important to determine the nutritional quality improvement of cowpea sprout flour due to elicitation treatment through the determination of nutrient compounds such as moisture, total mineral, fat, protein, as well as carbohydrate contents. The nutrition quality of cowpea sprout flour prepared with and without elicitation was presented in Table 1.

The result in Table 1 showed that germination with elicitation treatment proved capable to improve the nutritional quality of cowpea sprout flour, indicated by increasing levels of protein and total minerals, whereas fat and carbohydrate contents decreased. Germination has an impact on nutritional quality improvement by increasing protein and mineral content, as well as decreasing the fat and carbohydrate contents of cowpea flour (Owuamanam *et al*., 2013). The protein content elevation during legumes germination is possible due to the mobilization of storage nitrogen to produce the nutritionally high-quality proteins needed for the germination process, enzymes synthesis, the synthesis of newly-formed protein during germination, and a compositional change following the degradation of other constituents (Joshi and Varma, 2016; Xu *et al*., 2019). The enhancement of the total minerals during germination might be attributed to the leaching of anti-nutritional compounds with a mineral-bound ability. Germination helps reduce phytic acids known as anti-nutritional compounds with the ability to bind minerals, therefore promote to mineral availability increased (Nkhata *et al*., 2018). The fat and carbohydrate content reduction is related to enzymatic hydrolysis of both compounds during germination to fulfil energy for the developing embryo (Kajla *et al*., 2017; Joshi and Varma, 2016). The fat reduction is also attributed to the lipids used for the respiration process during germination (Nkhata *et al*., 2018). The nutrition quality improvement observed in the elicited cowpea sprout flour was related to the higher energy requirement to stress adjustment induced by elicitation. This impact on higher reduction in carbohydrate and fat levels due to the carbohydrate and fat hydrolysis to fulfil the energy requirements. The application of biotic and abiotic elicitors during germination induces physiological changes and stimulates the defence system by modifying the metabolism and adjusting to existing conditions that allow the modification of the nutrient composition (Baenas *et al*., 2014). The higher protein and mineral levels in elicited cowpea sprout flour might be also related to the compositional change following the degradation of fat and carbohydrate (Fouad and Rehab, 2015; Xu *et al*., 2019).

The NaCl elicitation induces an improvement in protein and total mineral contents of 4.27% and 4.78%, as well as a decrease in fat and carbohydrate levels by 1.13% and 1.94%. Enhancement in protein and total

Table 1. Nutritional quality of cowpea sprout flour prepared with and without elicitation.

Cowpea sprout flour preparation	Moisture (%)	Protein (% db)	Total mineral (% db)	Fat (% db)	Carbohydrate (% db)
NaCl elicitation	8.45±0.02^{b}	22.45±0.02^{b}	3.07±0.01^{b}	10.53±0.01^{a}	54.73±0.02^{b}
Na-alginate elicitation	8.54±0.04^{b}	22.62±0.10^{b}	3.13±0.02^{c}	10.52±0.02^{a}	54.39±0.12^{a}
Without elicitation	8.31±0.07^{a}	21.52±0.27^{a}	2.93±0.03^{a}	10.65±0.04^{b}	55.81±0.26^{c}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

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minerals levels, and reduction in fat and carbohydrate contents of cowpea sprout flour caused by Na-alginate elicitation reach 5.06%, 6.83%, 1.22%, and 2.54%, respectively. Na-alginate elicitation produces higher total minerals and lower carbohydrate contents than NaCl elicitation (Table 1). It is indicated that Na-alginate elicitation generates higher stress conditions than NaCl elicitation. This was supported by the higher total phenolic content (TPC) and total flavonoid content (TFC) of Na-alginate elicited cowpea sprout flour (Tabel 2). Higher stress levels induce higher secondary metabolite synthesis such as phenolic and flavonoid compound biosynthesis as the stress response (Ariviani *et al*., 2021; Rajendra *et al*., 2019). As mentioned above, it needed much higher energy to overcome the higher stress levels. The main energy was fulfilled from carbohydrate metabolism. Therefore, higher stress levels lead to higher carbohydrate level reduction. Thus, the Na-alginate elicited cowpea sprout flour exhibited lower carbohydrate levels than the NaCl elicited. Higher total mineral levels of Na-alginate elicited cowpea sprout flour observed as the consequence of the compositional change following the lower carbohydrate levels.

3.2 Antioxidant capacity of cowpea sprout flour

Table 2 exhibits that cowpea sprout flour has significantly lower levels of TPC (total phenolic content), TFC (total flavonoids content), RP (reducing power), and TEAC (trolox equivalent antioxidant capacity) rather than the elicited cowpea sprout flour. Elicitation is an effective strategy to increase bioactive antioxidant compounds including the phenolic and flavonoid compounds in legumes so that the antioxidant activities are elevated (Baenas *et al*., 2014; Peñas *et al*., 2015). The previous studies reported increasing total phenolic content of lentils (Peñas *et al*., 2015; Świeca, 2015), pigeon pea (Ariviani *et al*., 2020c), and total flavonoids levels of cowpea (Rajendra *et al*., 2019), due to elicitation treatment during legumes germination.

Cowpea sprout flour prepared with NaCl and Na-alginate elicitations showed higher levels of both TPC and TFC than that non-elicited cowpea sprout flour. The TPC and TFC of the flour with NaCl elicitation reach 7.89% and 11.29% higher compared to the non-elicited cowpea sprout flour. While the TPC and TFC of the flour with Na-alginate elicitation reach 17.48% and 17.09% higher than non-elicited cowpea sprout flour. Na-alginate elicited cowpea sprout flour exhibited higher improvement both in TPC and TFC. This result was related to the higher stress induces by Na-alginate elicitation. As mentioned previously in the nutrition quality section, the higher stress level condition induces a higher defence response among others by higher production of phenolic and flavonoid compounds.

The enhancement of RP and TEAC on Na-alginate elicited cowpea sprout flour was significantly higher than that of the NaCl elicitation, reaching 7.19% and 16.53% (Na-alginate), 3.41%, and 8.24% (NaCl) respectively. The highest RP and TEAC of the Na-alginate elicited cowpea sprout flour observed in this study was related to the highest TPC and TFC. Phenolic and flavonoid compounds can act as radical scavengers and reducing agents (Rajendra *et al*., 2019; Ariviani *et al*., 2020b; Ariviani *et al*., 2021). Ariviani *et al*. (2021) stated that several studies reported a high correlation between TPC and RP, and also TPC and TEAC, among others in faba beans (*Vicia faba* L.), grass pea (*Lathyrus sativus*), and non-oil seed legumes, as well as in the NaCl elicited cowpea sprout (Ariviani *et al*., 2020b). The strong correlation between TFC and RP as well as TFC and ABTS radical scavenging activity were reported in lentils sprouts flour (Świeca, 2015), and black sesame seed (Ruslan *et al*., 2018).

3.3 The functional properties of cowpea sprout flour

Characterization of flour's functional properties is essential to deciding its application in the manufacturing of food products, and regulating the processing and storage (Dereje *et al*., 2020). Table 3 shows the functional properties which include water holding capacity (WHC), oil holding capacity (OHC), water absorption capacity (WAC), oil absorption capacity (OAC), emulsion stability (ES) of cowpea sprout flour prepared with NaCl and Na-alginate elicitation as well as without elicitation.

Based on the data in Table 3, the NaCl and Na-alginate elicitations were proved capable to improve the functional properties of cowpea sprout flour indicated by the higher WHC, OHC, WAC, OAC, and ES values than

Table 2. Antioxidant capacity of cowpea sprout flour prepared with and without elicitation.

Antioxidant capacity	NaCl elicited cowpea sprout flour	Na-alginate elicited cowpea sprout flour	Non-elicited cowpea sprout flour
TPC (mM GAE/100 g db)	18.00±0.085^{b}	19.60±0.767^{c}	16.68±0.352^{a}
TFC (mM QE/100 g db)	17.89±0.192^{b}	18.82±0.153^{c}	16.07±0.249^{a}
RP (mM AAEA/g db)	297.92±2.97^{b}	308.81±2.51^{c}	288.10±4.43^{a}
TEAC (mM TEAC/100 g db)	64941.79±494.46^{b}	60017.40±309.33^{c}	59998.71±91.29^{a}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

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Table 3. Functional properties of cowpea sprout flour prepared with and without elicitation.

Flour Functional Properties	NaCl elicited cowpea sprout flour	Na-alginate elicited cowpea sprout flour	Non-elicited cowpea sprout flour
WHC (% db)	76.85±0.20^{c}	74.80±0.34^{b}	70.97±1.00^{a}
OHC (% db)	82.21±0.32^{b}	84.90±0.23^{c}	74.48±0.33^{a}
WAC (% db)	101.66±0.64^{c}	94.31±0.44^{b}	85.47±0.80^{a}
OAC (% db)	125.56±2.11^{b}	127.70±2.72^{b}	119.61±1.11^{a}
Emulsion Stability (% db)	58.26±3.16^{b}	59.23±1.58^{b}	50.89±1.57^{a}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

the flour prepared without elicitation. The WHC of flour describes as the amount of water retained within the protein matrix of flour which determines the functional capacity in thickening and food formulation (Enujiugha *et al*., 2003). Moreover, WAC represents the capacity of flour to associate with water under a restrictive water supply which reflects the capacity of flour to absorb water and swelling to enhance food uniformity. WAC is very important in various food products such as soups, dough, and baked products (Chandra *et al*., 2015; Dereje *et al*., 2020). The increase of WHC and WAC could be beneficial in food products that need to absorb water without protein dissolution thus obtaining body thickening and viscosity such as sausages, custards, and dough (Benítez *et al*., 2013). The OAC and OHC of legume flour are useful for retaining flavor and improving the mouthfeel of food products, while ES is important in various applications in food systems such as frozen desserts, cakes, and coffee whiteners (Elkhalifa and Bernhardt, 2010).

The functional properties of legume flour are influenced by its protein content. Higher protein levels produce higher levels of WHC (Sosulski and McCurdy, 1987), WAC (Elkhalifa and Bernhardt, 2010), OAC (Sosulski and McCurdy, 1987), and ES (Elkhalifa and Bernhardt, 2010). Protein has both hydrophilic and hydrophobic nature, and therefore, it can interact with water and oil in foods (Chandra *et al*., 2015) thus increasing WAC, WHC, OAC, OHC, and ES. The Increasing in WAC, OAC, WHC, and OHC has been observed due to the lower fat content (Sathe *et al*., 1982; Nguyen *et al*., 2015). Lower fat increases the exposure of water binding sites on the side chain group previously blocked in a lipophilic environment, thus increasing both WAC and WHC (Sathe *et al*., 1982). Moreover, the lower fat content increases the consumption of oil in legume flour structure, thus increasing OHC and OAC (Vandarkuzhali and Sangeethaetha, 2016). The results in Table 1 showed that elicited cowpea sprout flours have a higher protein content as well as lower fat content compared to the non-elicited cowpea sprout flour. Therefore, it has been observed that elicited cowpea sprout flours have significantly higher WAC, OAC, WHC, OHC, and ES than non-elicited cowpea sprout flour. Ariviani *et al*. (2020c) showed a similar result in pigeon pea sprouts with and without NaCl elicitation.

The WHC and WAC improvement of cowpea sprout flour were 8.27% and 18.84 %, 5.40%, and 10.35%, due to the NaCl and Na-alginate elicitation, respectively. The NaCl elicitation produces flour with higher WAC and WHC values than Na-alginate elicitation, although the protein and fat contents of both flours were not significantly different (Table 1). At low NaCl concentrations, the Na^{+} and Cl^{-} ions combine with charged protein molecules and reduce the water molecule around proteins thereby increasing the WAC and WHC of flour (Hu *et al*., 2017). Na-alginate elicitation showed higher OHC improvement (13.99%) than that of NaCl elicitation (10.38%). Limón *et al*. (2014) and Peñas *et al*. (2015) indicated that there was a modification of the protein profile due to the salt elicitation but a lack of modification was observed in biotic elicitation. Protein profile modification due to NaCl elicitation, results in higher content of amino acids with a polar side chain (R). These polar side chains hindered the interaction of the hydrophobic group with fat, thus decreasing OHC of the NaCl elicited cowpea sprout flour. Both cowpea sprout flour prepared with NaCl and Na-alginate elicitations produces similar improvement of OAC and ES reach to 4.98% - 6.76% and 14.46 - 16.36%, respectively. The OAC of cowpea sprout flour reached 119.61±1.11% and enhanced to 125.56±2.11% and 127.70±2.72% on the NaCl elicited and Na-alginate elicited cowpea spouts flour, respectively. While, the ES of cowpea sprout flour reached 50.89±1.57% and improved to 58.26±3.16% and 59.23±1.58% on the NaCl elicited and Na-alginate elicited cowpea spouts flour, respectively.

4. Conclusion

Elicitation using 50 mM NaCl and 250 ppm Na-alginate as biotic and abiotic elicitor has proven as an alternative simple strategy for improving nutrition quality, elevating antioxidant capacity (TPC, TFC, RP, and TEAC), as well as flour functional properties (WHC, OHC, WAC, OAC, ES) of cowpea sprout flour. The NaCl elicitation enhances protein and total minerals

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levels, WHC, WAC, OHC, OAC, ES, TPC, TFC, RP, and TEAC, and decreases the levels of fat and carbohydrate of cowpea sprout flour by 4.27%, 4.78%, 8.27%, 18.94%, 10.38%, 4.98%, 14.46%, 7.89%, 11.29%, 3.41%, 8.24%, 1.13%, and 1.94%, respectively. Whereas, Na-alginate elicitation induces increased protein and total mineral levels, WHC, WAC, OHC, OAC, ES, TPC, TFC, RP, and TEAC, and reduction of the levels of fat and carbohydrate of cowpea sprout flour by 5.06%, 6.83%, 5.40%, 10.35%, 13.99%, 6.76%, 16.39%, 17.48%, 17.09%, 7.19%, 16.53%, 1.22%, 2.54%, respectively. Na-alginate elicited cowpea sprout flour exhibited a higher improvement in OHC, nutrition quality, and antioxidant capacity than NaCl elicited cowpea sprout flour. On the other hand, NaCl elicitation produces a higher enhancement of WHC and WAC of the flour rather than Na-alginate elicitation. These results provide an alternative strategy to develop legumes as functional food ingredients.

Conflict of interest

The authors declare no conflict of interest.

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