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Mixed seeds juice with high antioxidant capacity and digestive enzyme activity and its application

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Abstract To investigate the synergistic and combined effects of a seed–vegetable combination, oilseed crops (safflower seed, evening primrose seed, and sesame seed) and cereals (whole buckwheat grain) were extracted with carrot using a low-speed juice extractor. Nutrient composition showed that the mixed seeds juice provided a well-balanced diet to supplement nutrient deficiency. Four assays of antioxidant activities showed that all seed juices exhibited higher activities than carrot juice. Similarly, mixed seeds juice displayed the highest α-amylase and protease activities, implicating the formulation in the improvement of the relatively low digestibility of seed. These results indicated that juice extraction significantly ($p<0.001$) influenced the nutrient contents, antioxidant activities, and digestion enzyme activities of seed juice. The synergistic and combined interactions of seeds and vegetables may be beneficial in enhancing biological functions and result in a well-balanced diet.

Keywords: juice, mixed seeds juice, antioxidant activity, nutritional composition, enzyme activity

Introduction

The growing global interest in sustained good health has spurred the consumption of fresh juices prepared using raw materials, including vegetables, fruits, cereals, and legumes. The consumption of fresh juices avoids the destruction of many nutrients that occur during heating in food processing. On the basis of the consumption of different food categories having synergistic, additive, and antagonistic effects on biological activities, various diets have been explored (1).

Safflower seeds contain significant amounts of important nutrients, polyunsaturated fatty acids, and polyphenol compounds. Moreover, the safflower hulls rich in *N*-feruloylserotonin, *N*-(*p*-coumaroyl) serotonin, and polyphenol compounds have antioxidant activities (2). However, safflower meal is being exclusively used as animal feed. Its bitter taste and reported cathartic activity precludes it from being utilized for human food (3). Buckwheat grains are important source of minerals and polyunsaturated fatty acids. In addition, buckwheat hulls have phenolic compounds and antioxidant activities comparable to those of buckwheat flour (4,5). Because of these beneficial biological effects, buckwheat has prompted the recommended intake of whole buckwheat grain without removing the hull. Evening primrose seed and sesame seed are enriched in unsaturated fatty acids. Sesame seeds contain sesamin and sesamolin, and then the two phenolic antioxidants, sesamol and sesaminol, are formed during sesame oil refinement. Both of these substances belong to lignans and reportedly lower cholesterol and alleviate hypertension (6). Evening primrose seeds have been used in wound healing and treatment of the Alzheimer disease owing to useful unsaturated fatty acids content (7).

Hulls possess potential biological activities similar to those of seeds and cereals and thus have a dietary value. However, they have been poorly utilized as a food source due to digestive problems. To solve this problem, seed juices were extracted with carrots to help increase the digestibility, functionality, and palatability of seed, particularly for the seeds with thick hulls and fibrous husks, using a low-speed juice extractor. Carrot is one of the most economically important vegetable crops in the world (8). Owing to the crunchy texture and sweetness of carrots, it can be employed in some fruit-like roles. Choi *et al*. (9) have demonstrated that carrot has the highest α-amylase activity among 12 different vegetables and fruits juice. From the above-mentioned nutritional viewpoint, carrot was chosen as the vegetable for increasing the digestibility, functionality, and palatability of seeds during seed juice preparation.

On the other hand, the nutrient composition and biological activity of oilseed crops and cereals extracted using a low-speed juice extractor have not been investigated to date. Thus, this study aims at evaluating the potential of mixed seeds juice as a source of high nutrition compositions, antioxidant activities, digestive enzyme activities, and a well-balanced diet by comparison to individual juices.

Materials and Methods

Sample preparation and particle size analysis Carrots were purchased from a local farmers market at Busan, Korea. The safflower (*Carthamus tinctorius*) seeds, evening primrose (*Oenothera odorata*) seeds, whole buckwheat (*Fagopyrum esculentum*) seeds, and sesame (*Sesamum indicum*) seeds were purchased from Armdaun-Nongwon (Seoul, Korea). The washed carrots (*Daucus carota*) were peeled and sliced and were extracted using an Angelia 8,000 stainless-steel juice extractor (Angel Co., Ltd., Busan, Korea). The seed juices were extracted along with carrot (300 g) and classified as follows: only carrot (carrot juice), carrot and safflower seed (20 g, safflower seed juice), carrot and evening primrose seed (20 g, evening primrose seed juice), carrot and buckwheat (20 g, buckwheat juice), carrot and sesame seed (20 g, sesame seed juice), and carrot and four types of seeds (20 g, mixed seeds juice). Juice yield (%) was calculated as [weight of the juice (g)/weight of raw materials (g)]×100. Particle size was measured using a model LS 13 320 particle size analyzer (Beckman Coulter Inc., Palo Alto, CA, USA).

Sensory evaluation Initially and periodically, sensory characteristics of all types of juice were evaluated for different sensory attributes by a panel of 23 panelists. Sensory attributes, such as color, aroma, taste, and overall acceptance for all seed juices, were assessed using four-point hedonic scales. The following hedonic scale was considered: 1=disliked extremely, whereas 4=liked extremely, as reported by Iwe (10). The seed juices were coded with a number and randomly served to panelists to avoid any bias.

Analysis of mineral composition Minerals were analyzed using the method described by Korean Food Standards Codex (11) with some modifications. Macro-elements (Ca, Mg, P, Fe, and Na) and micro-elements (Zn, Mn, and Cu) were determined using an inductively coupled plasma-optical emission spectrometer (Optima 8300; Perkin Elmer Inc., Waltham, MA, USA), and selenium was determined using an inductively coupled plasma-mass spectroscopy (iCAP Q; Thermo Fisher Scientific, Waltham, MA, USA).

Analysis of vitamin composition Vitamins A and E were determined according to Korean Food Standards Codex (11). On the other hand, vitamin B in the filtrate was determined by modifying a previously described method (12). Vitamin compositions were analyzed via high-performance liquid chromatography (Agilent 1200; Agilent, Santa Clara, CA, USA).

Analysis of fatty acid composition Fatty acid methyl esters (FAMEs) of seed juice were prepared by Korean Food Standards Codex (11) and analyzed using a gas chromatograph (GC-7890; Agilent) with flame ionization detection.

Preparation of methanol extracts of seed juices Seed juices were extracted five times with methanol at room temperature for 2 h. Each extract was filtered through a filter paper and diluted by 50% or 10% for analysis.

Determination of antioxidant activities The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of each seed juice extract was measured based on a previously described method (13). DPPH solution was added to each extract, followed by incubation in the dark for 30 min at 37°C. The absorbance was measured at 517 nm. 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation solution (14) was diluted with distilled water to obtain an absorbance of 0.70±0.01 at 734 nm. The dilute solution was added to the seed juice extract. After 1 h in the dark, the absorbance was measured at 734 nm. The superoxide dismutase (SOD)-like activity of seed juice extracts was determined using a commercially available SOD assay kit (Dojindo Molecular Technologies, Kumamoto, Japan). All procedures were complied with the manufacturer's instructions. The nitric oxide (NO) radical scavenging activity of the seed juice extracts were measured based on a previously described method (15). Sodium nitroprusside solution was mixed with an equal volume of seed juice extract and incubated at room temperature for 180 min. Griess reagent was added at room temperature for 10 min, and the absorbance was measured at 542 nm.

Determination of digestive enzyme activities The α-amylase activity of each seed juice extract was determined by a modified 3,5-dinitrosalicylic acid (DNS) method (16). One unit of activity was defined as the amount of enzyme that produced 1 μmol of maltose per min under assay condition. Protease activity was determined using casein as the substrate (17). One unit of activity was defined as the quantity of enzyme that liberates the equivalent of 1 μg of L-tyrosine per min under the assay condition.

Statistical analyses The data represented a mean value and standard deviation (SD) of triplicate measurements. Statistical analyses were performed using the Student's *t*-test and Duncan's multiple range test functions of SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Significance was defined as $p<0.001$ or 0.05.

Results and Discussion

Determination of optimal amount of seeds The analysis of juice yield and particle size by extraction with different amount of input seeds (0, 10, 20, 30, and 40 g) was conducted to determine the optimal seed amount (data not shown). Seeds were extracted along with carrot (300 g). Juice yield of 20 g seeds (77.33%) was the highest. The yield of seed-free carrot juice (64.97%) was the lowest. Juice yield improved as the seed content increased with 10 and 20 g seeds; whereas, it decreased when 30 and 40 g seeds were used. Similarly, particle size of juice containing 20 g seeds (10.97 μm) was the smallest, while carrot juice (26.32 μm) was the largest. The mean sensory score of mixed seed juice samples is summarized in Table 1. All scores obtained were in the range 1.5–3.1, and 20 g of seeds showed slightly higher than the others in their overall acceptance although there was no significant difference (p>0.05) between the amount of seeds (10, 20, and 30 g). However, 40 g of seeds received low marks in nearly every category. When these findings are taken together, the optimum seed amount was 20 g per carrot.

Table 1. Sensory score of mixed seeds juice with different amount of seeds (per 300 g of carrot)

Amount of seeds[1)]	Color	Aroma	Taste	Overall acceptance
Each 10 g	$2.8^{b2)}$	2.4^{ab}	2.5^{ab}	2.7^{b}
Each 20 g	3.1^{b}	2.7^{b}	2.9^{b}	3.0^{b}
Each 30 g	2.6^{b}	3.0^{b}	2.7^{b}	2.6^{b}
Each 40 g	1.5^{a}	1.9^{a}	2.0^{a}	1.7^{a}

[1)]Seeds of input are safflower seed, evening primrose seed, buckwheat, and sesame seed.
[2)a,b]Mean with different superscripts within a column are significantly different, and the same superscripts do not significantly differ at p<0.05.

Comparison of yield of seed juices Variance in the juice yield by the addition of the different kinds of seeds was determined (data not shown). Evening primrose seed juice had the highest extraction yield, (79.91%) followed by mixed seeds juice (76.84%). The yield of all seed juices was statistically higher than that of carrot juice, except for sesame seed juice (p<0.001). The results suggested that the juice yield increased in a seed-dependent manner. The pH value of all seed juices was the same (pH=6.0±0.1).

Determination of mineral composition The mineral element analysis in seed juices is summarized in Table 2. Among the macro-elements, K was the most prevalent in all seed juices. Mixed seeds juice was most enriched in mineral elements, while carrot juice was least enriched, except for Na. The Mg and P contents of mixed seeds juice were 695.63 and 1,061.00 mg/kg, respectively, which were higher than those of the other seed juices. Carrot juice contained 198.23 mg/kg of Na, which was similar to that in seed juices (175.77–206.57 mg/kg). Micro-elements (Se, Cu, Mn, and Zn) known to exhibit antioxidant activities were generally increased by addition of seeds. The micro-elemental composition of individual seed juices was lower than that of mixed seeds juice. Mixed seeds juice was enriched in Se, an essential component of glutathione peroxidase, by 5.3-fold compared with carrot and buckwheat juice, 3.2-fold compared with safflower seed juice, and 2.7-fold compared with the evening primrose and sesame seed juices. The reported Se content of safflower seed (0.0090–0.0261 μg/mL) was smaller than that of the plant leaf and root (18). Mixed seeds juice showed a similar Se content (0.016 mg/kg), with less Se detected in safflower seed juice (0.005 mg/kg) than previously reported (18). Another study reported that K and Ca are the most abundant minerals in safflower seed, which is consistent with our results (19). However, contrary to the previous findings, our results indicated that P was the third most abundant mineral in safflower seed juice (9.7% of the total mineral composition). This difference could have been due to the addition of carrot, which is enriched in P.

Determination of vitamin contents The vitamin contents in seed

Table 2. Mineral and vitamin compositions (mg/kg) of juice samples[1)]

			Carrot juice	Safflower seed juice	Evening primrose seed juice	Buckwheat juice	Sesame seed juice	Mixed seeds juice
Minerals	P		332.50±3.95	411.33±3.35	518.50±6.20	399.93±3.59	530.57±5.35	1061.00±20.78
	Mg		74.37±0.21	213.30±0.44	266.70±0.85	210.07±1.68	294.60±4.10	695.63±15.99
	Ca		284.53±4.90	426.87±1.18	572.50±8.70	370.23±3.14	764.83±20.41	788.73±25.92
	K		2420.33±9.24	2986.67±7.09	3274.33±4.93	3118.00±20.07	2711.33±31.79	3233.67±62.98
	Zn		2.77±1.24	8.59±0.15	5.85±0.05	6.05±0.19	10.23±0.19	22.80±0.48
	Mn		0.73±0.02	2.47±0.03	6.60±0.04	2.83±0.02	2.59±0.23	11.46±0.26
	Fe		5.00±0.53	9.89±0.13	9.94±0.96	6.60±0.03	11.60±1.17	38.63±0.44
	Na		198.23±1.63	188.77±2.58	205.07±1.55	206.57±1.40	175.77±3.09	182.53±4.55
	Cu		0.40±0.01	1.20±0.01	0.80±0.00	0.90±0.00	1.60±0.01	2.80±0.02
	Se		0.003±0.001	0.005±0.001	0.006±0.003	0.003±0.004	0.006±0.004	0.016±0.010
Vitamins	β-Carotene		148.46±3.82	129.57±6.70	139.58±8.11	87.67±2.48	104.13±6.13	89.87±8.29
	B1		0.27±0.01	0.55±0.00	0.08±0.00	0.44±0.05	0.68±0.01	0.48±0.02
	B2		0.09±0.01	0.22±0.021	0.08±0.02	0.10±0.01	0.07±0.01	0.13±0.00
	B3		0.19±0.01	0.19±0.03	0.14±0.01	0.15±0.01	ND	0.07±0.02
	B5		0.78±0.04	1.05±0.04	0.81±0.05	0.83±0.08	0.59±0.04	0.72±0.05
	B6		ND[2)]	ND	0.14±0.00	ND	ND	0.09±0.01
	E	α	6.63±0.24	11.01±0.55	9.60±0.17	5.61±0.32	4.56±0.06	15.05±0.62
		γ	ND	ND	0.50±0.01	0.44±0.04	2.45±0.19	3.07±0.11

[1)]Mean±standard deviation of data obtained from three independent experiments.
[2)]ND, Not detected.

juices are summarized in Table 2. The β-carotene content of carrot juice (148.46 mg/kg) was the highest. Mixed seeds juice contained less β-carotene as compared with individual seed juices. This indicates that β-carotene is negligible in seeds and that carrot can be utilized to enhance seed juice nutrition by supplying vitamins instead of seeds. Vitamin B1 was the highest in sesame seed juice (0.68 mg/kg) and safflower seed juice (0.55 mg/kg), with the lowest amount in carrot juice (0.27 mg/kg) and evening primrose seed juice (0.08 mg/kg). Mixed seeds juice contained 0.48 mg/kg of vitamin B1, which was the average component of the total seed juice. Vitamin B6 was detected only in evening primrose seed juice and mixed seeds juice. The vitamin E content of β-tocopherol and δ-tocopherol were not observed in any juice. But α- and γ-tocopherol, which have profound biological activities, were most prevalent in mixed seeds juice at 15.05 and 3.07 mg/kg, respectively. γ-Tocopherol was detected only in the sesame seed juice, evening primrose seed juice, buckwheat juice, and mixed seeds juice.

Pyo *et al*. (20) reported that total tocopherol content of evening primrose seed oil was 721 mg/kg, which comprised 34.0% α-tocopherol and 66.0% γ-tocopherol. Vitamin E of sesame seed oil predominantly composed γ-tocopherol, with δ-tocopherol as the remainder; α- and β-tocopherol are undetectable (21). Our results indicated the synergistic and combined effects of all seed juices with the addition of carrot as a means of supplementing α-tocopherol.

Determination of fatty acid composition The fatty acid composition in seed juices is summarized in Table 3. All seed juices were enriched in linoleic, which is an essential fatty acid for maintenance of health, including reduction of cholesterol (22). The linoleic acid content of mixed seeds juice (3.436 g/100 g) was elevated by 16.2-fold compared with carrot juice, 14.6-fold compared with buckwheat juice (0.235 g/100 g), approximately 3.2-fold compared with the safflower (1.060 g/100 g) and evening primrose seed juices (1.144 g/100 g), and 2.3-fold compared with sesame seed juice (1.509 g/100 g).

DPPH radical scavenging activity As shown in Fig. 1A, the 92.46% activity of undiluted mixed seeds juice was the highest, followed by evening primrose seed juice (89.52%). This activity was significantly higher than that of 50-μg/mL butylhydroxytoluene (BHT, 79.33%) and 10-μg/mL L-ascorbic acid (52.34%) (p<0.001). As compared with carrot juice, the seed juices exhibited enhanced activity. In particular, the highest increase was founded in mixed seeds juice (3.80-fold), followed by buckwheat and evening primrose seed juice (3.67-fold greater than carrot juice). These results indicate a strong combined effect on DPPH radical scavenging by the combination of seeds. Safflower seed juice inhibited DPPH radicals by 66.9%. Other studies reported a rate of 36.2% for hot water extracts of safflower seed (1.0 mg/mL) (23). Furthermore, the rate of 61.16% for sesame extract at 1.0 mg/mL was higher than 37.21% for sesame seed juice found in this study. This difference on DPPH radical scavenging activity could reflect conditions, including cultivars, growing region, maturity stage and methods of storage and the use of different extracting solvents.

ABTS radical scavenging activity The activity of the juices increased in a dose-dependent manner (Fig. 1B). The highest scavenging activity was observed for mixed seeds juice, followed by evening primrose seed juice, buckwheat juice, sesame seed juice, and carrot juice. Mixed seeds and evening primrose seed juices displayed scavenging activities of 91.83 and 74.83%, respectively, which were higher than the positive controls (100 μg/mL). Yu *et al*. (23) reported that the hot water extract of safflower seed at 1.0 mg/mL displayed 15.3% ABTS radical scavenging activity, which was lower than the 18.18% activity of 50% safflower seed juice.

SOD-like activity To investigate the antioxidant capacity of juices, SOD-like activity was measured (Fig. 1C). All juices exhibited pronounced SOD-like activity, which was significantly higher than 67.20% by the 200-μg/mL L-ascorbic acid positive control (p<0.001). In particular, mixed seeds, evening primrose seed, and safflower seed

Table 3. Fatty acid compositions (g/100 g) of juice samples[1)]

Composition	Carrot juice	Safflower seed juice	Evening primrose seed juice	Buckwheat juice	Sesame seed juice	Mixed seeds juice
Palmitic acid	0.063±0.001	0.133±0.001	0.157±0.001	0.090±0.001	0.420±0.002	0.652±0.002
Stearic acid	0.006±0.000	0.040±0.001	0.034±0.001	0.010±0.001	0.248±0.003	0.322±0.003
Oleic acid	0.029±0.001	0.168±0.002	0.123±0.002	0.117±0.002	1.517±0.012	1.755±0.009
Linoleic acid	0.212±0.005	1.060±0.007	1.144±0.012	0.235±0.003	1.509±0.012	3.436±0.014
Linolenic acid	0.025±0.001	0.016±0.001	0.014±0.001	0.024±0.001	0.029±0.001	0.041±0.001
Arachidic acid	ND[2)]	ND	ND	ND	0.024±0.001	0.042±0.001
Gadoleic acid	ND	ND	ND	ND	0.006±0.001	0.019±0.000
Behenic acid	ND	ND	ND	ND	0.005±0.001	0.013±0.001
Lignoceric acid	ND	ND	ND	ND	ND	0.007±0.000
Saturated fatty acid	0.069±0.000	0.173±0.000	0.191±0.000	1.000±0.000	0.696±0.002	1.036±0.001
Unsaturated fatty acid	0.265±0.002	1.244±0.002	1.282±0.004	0.376±0.001	3.061±0.006	5.251±0.006
Total fatty acid	0.334±0.001	1.418±0.001	1.473±0.002	0.476±0.001	3.757±0.004	6.288±0.004

[1)]Mean±standard deviation of data obtained from three independent experiments.
[2)]ND, Not detected.

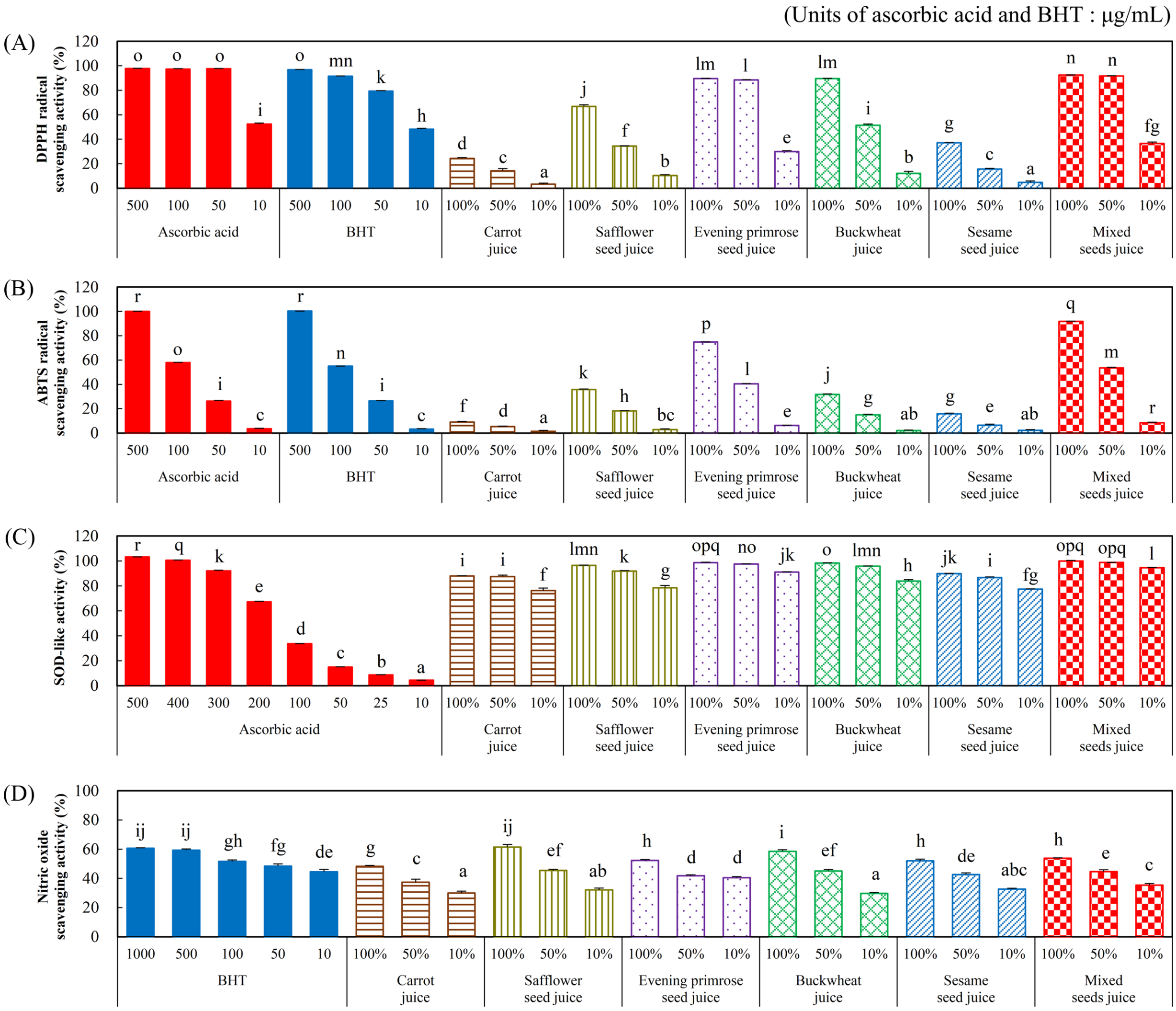

Fig. 1. Antioxidant activities of juice samples. (A) 1,1-diphenyl-2-picrylhydrazyl (DPPH), (B) 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), (C) superoxide dismutase (SOD)-like, and (D) nitric oxide (NO) scavenging activity. Data are mean±standard deviation (SD) of at least three replicates of juice samples. [a–r]Values not sharing common letter indicate significant difference from each other (p<0.001).

juices activated SOD by 99.87, 98.58, and 96.37%, respectively, which was higher than 92.00% by 300-µg/mL L-ascorbic acid. Sesame seed juice displayed a poor increase in the activity (89.82%), while the other seed juices improved SOD-like activation when used in combination with carrot. An even lower activity of sesame has been described (24). In contrast to carrot juice, the difference likely resulted from carrot because the activities of the four seed types showed poor activity, with the highest activity of 10.71% in evening primrose seed. These results implicate the importance of carrot in the antioxidant capacity as supplement to seeds lacking SOD-like activity.

NO radical scavenging activity As shown in Fig. 1D, the activity of all juices was increased in a dose-dependent manner. Safflower seed juice (61.40%) and buckwheat juice (58.48%) showed high activities, which significantly exceeded that of the 100-µg/mL BHT positive control (p<0.001). All other seed juices improved the NO radical scavenging activity via combination with seeds. The juice extract inhibits nitrite formation by directly competing with oxygen in the reaction with NO. This study proved that the all seed juices have more potent NO scavenging activity than the carrot juice.

Amylase activity The enzymatic degradation of starch in plants has been attributed to α-amylase in starch storage tissues. They have been particularly studied in seeds, where they are responsible for the breakdown of polysaccharide reserves. In the dry grains of cereals, there is generally a low level of β-amylase activity and significantly low α-amylase activity present in the starchy endosperm (25). Previous research on 12 vegetables and fruits reported α-amylase activity in carrot but not in apple, grape, tomato, or papaya (8).

As shown in Fig. 2A, the amylase activity of all seed juices was similar to that of carrot juice. There was no increase in seed juices compared with carrot juice (58.98 unit/mL). The high activity of all juices could be attributed to carrot juice. In contrast, the activity of

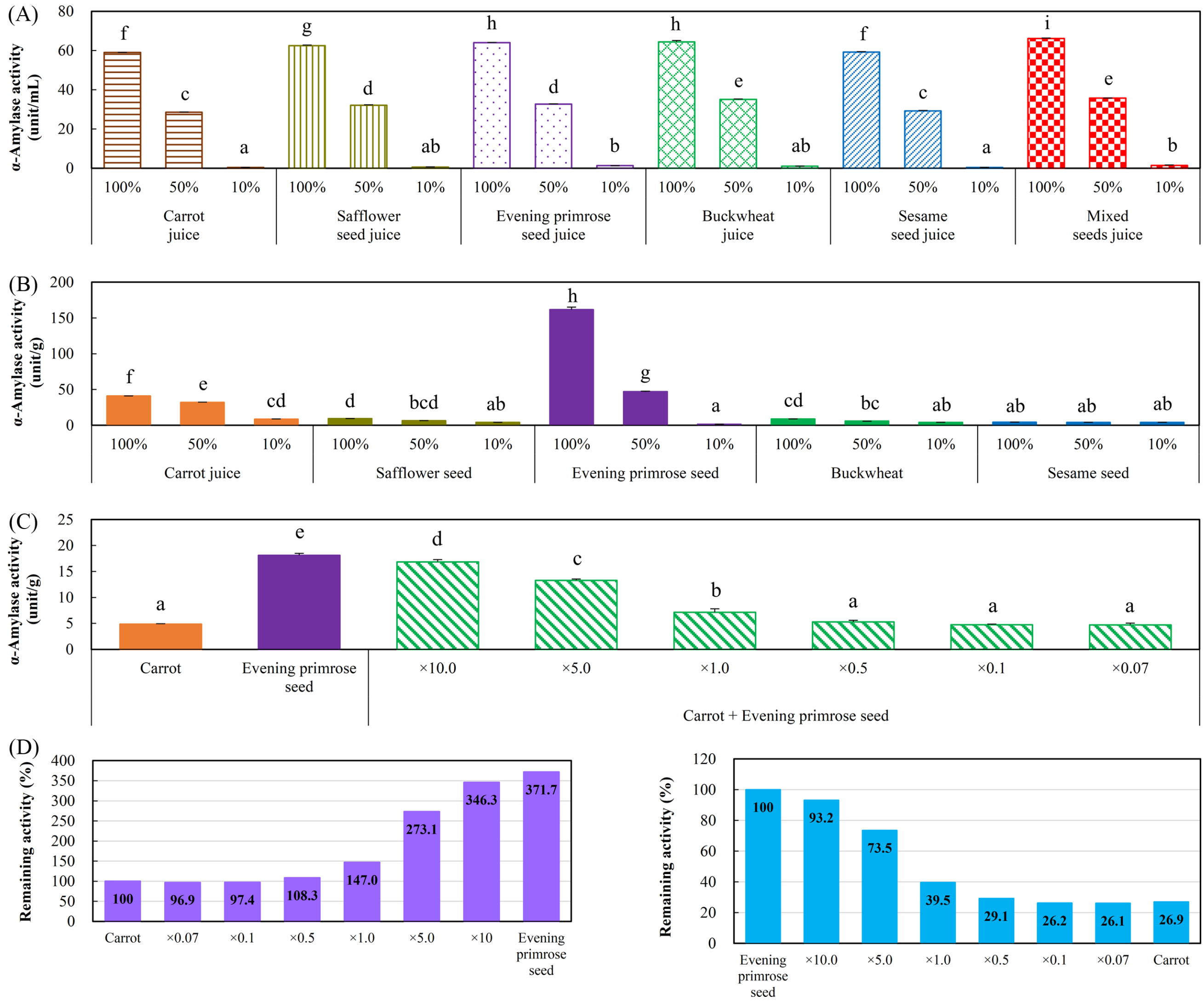

Fig. 2. α-Amylase activities of juice samples. (A) Mixture in carrot and seeds, (B) only seed juice or carrot juice, (C) evening primrose seed juice by mixing with different amount input carrot, and (D) the remaining activities of evening primrose and carrot juices. Data are mean±SD of at least three replicates of juice samples. [a–i]Values not sharing common letter indicate significant difference from each other (p<0.001).

seed itself exhibited very low levels of 0.25–7.12 unit/mL. From this result, it may possible to consider that there may nearly be absence of amylase in the seeds. To further study amylase activities in seeds, each seed juice extract was prepared in the absence of carrot and extracted with methanol, as described in Materials and Methods. Contrary to the amylase activities of seed juices shown in Fig. 2A, evening primrose seed showed the highest activity, followed by carrot, safflower seed, buckwheat seed, and sesame seed (Fig. 2B). Figure 2C shows the changes in amylase activities of evening primrose seed juice by mixing with different amounts of input carrot. As shown in Fig. 2C, the amylase activity of evening primrose seed juices after the addition of carrot was decreased in a dose-dependent manner. Although evening primrose seed alone showed 3.9-fold higher activity than carrot, as shown in Fig. 2D, the remaining activities of evening primrose seed juice at 0.1-fold and 0.07-fold input ratio were decreased to 97.4 and 96.9%, respectively. In addition, the remaining activity of evening primrose seed was 39.5% at 1.0-fold input ratio. This result indicates that the amylase activity of evening primrose seed was decreased with the addition of carrot. Nevertheless, all seed juices have high amylase activity due to carrot juice.

To date, this is the first report to evaluate the antagonistic effect of carrot on the amylase activities of seeds. However, the exact mechanism of the antagonistic action of carrot on the seed is not clear.

On the other hand, Elarbi *et al.* (16) reported that α-amylase purified from safflower-germinating seeds showed a minimum basal level at zero day of growth and maximum activity after five days of growth. Nevertheless, in this study, all the seed juices including safflower seed juice showed high activity due to carrot juice. Therefore, the ingestion of mixed seeds juice is expected to have health benefits among people who are currently prone to high

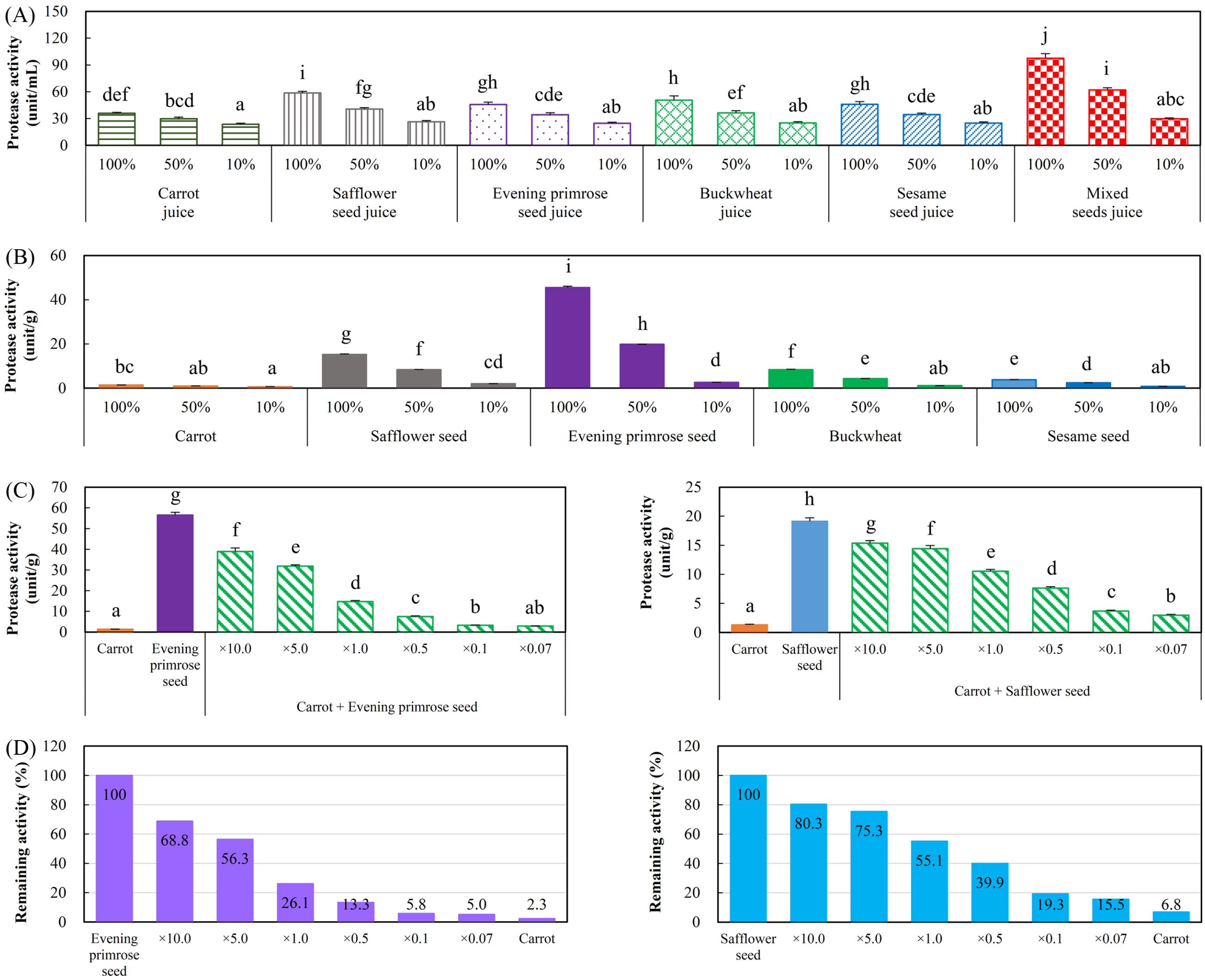

Fig. 3. Protease activities of juice samples. (A) Mixture in carrot and seeds, (B) only seed juice or carrot juice, (C) evening primrose seed or safflower seed juice by mixing with different amount input carrot, and (D) the remaining activities of the evening primrose or safflower seeds and carrot juices. Data are mean±SD of at least three replicates of juice samples. [a–j]Values not sharing common letter indicate significant difference from each other (p<0.001).

polysaccharide food intake.

Protease activity Proteases are essential in digestion as they break down food protein to liberate amino acids needed by the body. As shown in Fig. 3A, the protease activity of all the juices was increased in a dose-dependent manner, with the highest activity found in mixed seeds juice (97.50 unit/mL), followed by safflower seed juice (58.61 unit/mL), buckwheat juice (50.55 unit/mL), sesame seed juice (45.85 unit/mL), evening primrose seed juice (45.65 unit/mL), and carrot juice (35.84 unit/mL). In particular, mixed seeds juice displayed a 2.7-fold higher activity than carrot juice (p<0.001). The protease activity of all seed juices was higher than carrot juice. To further study protease activities in seeds, each seed juice extract was prepared in the absence of carrot and extracted with methanol, as described in Materials and Methods. As shown in Fig. 3B, evening primrose seed showed the highest activity, followed by safflower seed, buckwheat seed, sesame seed, and carrot. From this result, we can expect that the resultant synergistic activity of mixed seeds juice was originated from the combined effect of each seed juice. Among these seeds, the safflower and evening primrose seeds were considered as representative samples to further characterize the protease activity in more detail. As shown in Fig. 3C, the activities of the safflower and evening primrose seed juices were decreased in a dose-dependent manner by addition of carrot. Although the protease activity of evening primrose seed alone was 3.0-fold higher than that of safflower seed (Fig. 3B), as shown in Fig. 3D, the remaining activity of safflower seed after the addition of carrot was significantly increased compared with evening primrose seed. The remaining activities of the safflower and evening primrose seeds at 1.0-fold input ratio were 55.1 and 26.1%, respectively. Although the protease activity of evening primrose seed was decreased with the addition of carrot, the resulting protease activity was always higher than that of carrot under different amounts of input carrot. To the best of our knowledge, this is the first study that evaluates the protease activity of the safflower seed, evening primrose seed, buckwheat, and sesame seed.

Consequently, our results indicate a synergistic (i.e., mineral, vitamin E, and protease activity) and combined (i.e., unsaturated fatty acid, antioxidant activity, and α-amylase activity) effect on the nutrient content and antioxidant capacities of seed juices when oilseed crops, including sesame, evening primrose seed, and safflower seed, cereals such as buckwheat, and vegetables such as carrot, are extracted together. Moreover, the results suggest that combining seeds and vegetables improve digestive function. This biological and balanced nature of a diet could be enhanced by using the juice formulated with seed combinations that exhibit synergistic and combined interactions.

Disclosure The authors declare no conflict of interest.

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