



Effect of different nutraceuticals on phytochemical and mineral composition as well as medicinal properties of home made mixed vegetable pickles

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ABSTRACT

The present study was carried out to check the effect of different nutraceuticals on antioxidant activity, total phenolic contents, total flavonoid contents, antidiabetic activity, sensory evaluation and heavy metals of mixed vegetable pickles. The results were indicated that the P3 pickle sample which contained six medicinal plant powders showed the highest antioxidant activity ($87.56 \pm 0.02\%$), total phenolic contents (77.12 ± 0.01 mg GAE/100 g), total flavonoid contents (14.67 ± 0.06 QE mg/g) and antidiabetic activity ($IC_{50} = 25.89 \pm 0.07$ μ g/mL). The level of heavy metals was varied in all of the selected pickles but below permissible limit while according to consumer point of view all sample were accepted.

KEYWORDS: Nutraceuticals; mixed pickles; antioxidant activity; antidiabetic activity; heavy metals

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INTRODUCTION

Pickling is the oldest and useful method which is used for the preservation of food by anaerobic fermentation or immersion in the vinegar and resulting food is called pickle. The word pickle is derived from Dutch language "pekel" which mean brine. Pickle is fermented food which contains various vegetables (beans, carrot, mango, green tomatoes, pepper, cabbage etc.) and spices while additives are added for the taste improvement. Sweet, vinegar, salt and oil pickles are very common commercial manufacture varieties. Salt pickle contains fresh and pure materials and salt; it is preserved with 12-15%. Basically, lactic acid bacteria are used for the conversion of sugar into acid [1-3]. Salt plays main role in the fermentation process and is used to drawing out nutrients and water from the vegetables which are used as ingredients while salt is also used as substrate for the growth of lactic acid bacteria. In pickling process sugar is converted into acid (lactic acid) at acidic condition (approximately 4.6) and because of this acidic medium pathogen and aerobic spoilage microorganism's growth is inhibited. Due to pickling process texture and flavour of pickled vegetables are changed and after pickling show firm texture, translucent appearance and longer shelf-life [3-5]. Pickling process is used to preserve the vegetables for long time. Vinegar and vegetable oil is used as

pickling medium in Asia [6]. Pickles contain phytochemicals and minerals which come from ingredients (Vegetables and spices), therefore pickles are useful against different diseases such as cancer, inflammation, brain dysfunction, atherosclerosis. The bioactive compounds are phenolic acids mostly flavonoids and tannins which scavenge the free radicals which are harmful for health and cause different diseases [7-9].

MATERIALS AND METHODS

Chemicals and Ingredients

DPPH (2,2-diphenyl-1-picrylhydrazyl), quercetin, ascorbic acid, gallic acid, perchloric acid, nitric acid, hydrogen peroxide, distilled water, 3, 5-dinitrosalicylic acid (DNSA), folin-ciocalteu phenol reagent and all other chemicals and reagents which were used in all over the experiments were purchased from Merck (Darmstadt, Germany). All the chemicals used in research were of analytical grade.

Collection and Preparation of Food Material

Total four pickle samples were used in this study and samples were differentiated on the basis of medicinal plant powders. For the

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preparation of mixed vegetable pickles (carrot, green chili, lemon, bitter gourd and turnip), spices and mustard oil were purchased from local market of Faisalabad, Pakistan. While for value addition the plants (*Solanum nigrum*, *Fogonia indica*, *Ocimum tenuiflorum*, *Azadirachta indica*, *Mangifera indica* and *Moringa oleifera*) were also collected from Faisalabad, Pakistan and these plants were confirmed by Department of Botany, University of Agriculture, Faisalabad, Pakistan. The pickle sample which contained (*Solanum nigrum*, *Fogonia indica*, and *Ocimum tenuiflorum*) grounded powders classified as P1, pickle sample which contained (*Azadirachta indica*, *Mangifera indica* and *Moringa oleifera*) grounded powders denoted by P2, pickle sample contained (*Solanum nigrum*, *Fogonia indica*, *Ocimum tenuiflorum*, *Azadirachta indica*, *Mangifera indica* and *Moringa oleifera*) grounded powders classified as P3 while controlled was denoted by P4.

Recipe and Formulations

The mixed pickle samples were prepared by previously described assay with minor modification [5]. Firstly, vegetables were washed, peeled and trimmed into small pieces and added 3-4g potassium meta bi-sulfite and this process is known as sulphiting. After that vegetables were cooked for different times at different temperatures like green chili was cooked for 4-5 minutes at 33-40°C and slices of carrots were cooked for 8-10 minutes at 40-50°C in hot water. The cooking process is known as blanching and this process is used for the inhibition of enzymatic activity. After that spices were added and again cooked for 2 minutes and then medicinal plant powders were added and further cooked for 6-8 minutes in mustered oil. The pickles were stored in the jar at 25°C until use. All the samples were prepared by the same way but differentiated only on the basis of medicinal plant powders.

Analysis of Antioxidant Activity by DPPH free Radical Scavenging Assay

The antioxidant activity of mixed pickle samples was determined by using DPPH free radical scavenging assay as previously described with minor modifications [10].

Determination of Total Phenolic Contents

The total phenolic contents were determined by folin–ciocalteu colorimetric assay as previously described with minor modifications [10].

Determination of Total Flavonoid Contents

Total flavonoid contents were determined by using aluminium chloride assay as described previously with minor modification [1].

Alpha Amylase Inhibition Assay

Alpha amylase inhibition assay was used to determine antidiabetic activity of mixed pickle samples as previously described [10].

Determination of Heavy Metals By Wet Digestion Assay

The heavy metals were determined in pickles by using wet digestion assay as previously described [11]. Briefly, 1mL of each sample mixed with 10mL of concentrated HNO₃ and placed on a hot plate for 10 minutes at 60-70°C. After that mixtures were cooled at room temperature and then added 5mL of HClO₄ and again mixtures were placed on the hot plate at 60-70°C until white fumes evolved and then mixtures were cooled at room temperature. After that 5mL H₂O₂ was added in each sample mixture and finally diluted by mixing distilled water on desired volume and results were determined by using atomic absorption spectrophotometer. The results were expressed in parts per million (ppm).

Sensory Evaluation

Total six panellists between 20-50 years from University of Agriculture Faisalabad, Pakistan participated in this study. Total 25-point hedonic scales were used for determination of sensory attributes of pickles [12].

Statistical Analysis

All analysis and tests were repeated three times and obtained results were expressed as mean ± standard deviation. The analysis of variance was used to check significant difference in samples. Statistical significance was set at p<0.05 [13].

RESULTS AND DISCUSSION

Antioxidant Activity

The antioxidant activity of value added mixed pickle samples was assessed by free radical scavenging assay. The values of antioxidant activity were lies in the range of (87.56 ± 0.02 to 64.11 ± 0.01%). The highest inhibition (87.56 ± 0.02%) against free radicals was shown by P3 sample while lowest inhibition (64.11 ± 0.01%) was observed in control. The ascorbic acid was used as standard found to be (89.54 ± 0.03%). The significant level was p< 0.05. The detailed results are shown in Table 1. The results were compared with previous findings whereby the range of antioxidant potential of leaf mustard pickle was reported as 37.25-85.36%; while at 14-day preservation methanol extracts showed highest antioxidant activity 85.36% and ethanol extracts showed lowest antioxidant activity 37.25% at zero-day storage [13]. The present research provided interesting and very useful information that the mixed pickle which contained six medicinal plant powders (P3) showed excellent antioxidant potential as compared to others.

Total Phenolic Contents

The total phenolic contents were determined by folin–ciocalteu colorimetric assay which previously described. The total phenolic contents were lies in the range of (77.12 ± 0.01 to 43.09 ± 0.05 mg GAE/100 g). The highest phenolic contents (77.12 ± 0.01 mg GAE/100 g) were found in P3 pickle sample

while lowest phenolic contents (43.09 ± 0.05 mg GAE/100 g) were indicated by control. The details were discussed in Table 1. According to previous finding the results of phenolic contents of 10 vegetables pickles after 15-day pickling were lies in the range of 12.23 ± 0.98 - 64.25 ± 7.31 mg GAE/100g FW. The total phenolic contents of cucumber, carrot, sneak melon, green beans, tomato, green paper, white cabbage, cauliflower and chili pepper was 12.23 ± 0.98 , 14.18 ± 1.32 , 15.36 ± 1.25 , 18.32 ± 2.11 , 20.02 ± 2.15 , 20.98 ± 3.41 , 48.25 ± 6.82 , 49.56 ± 7.52 and 64.25 ± 7.31 respectively [5]. Our research provided motivating information about nutraceuticals and according to our results nutraceuticals are beneficial for human health and nutraceuticals gives much better results if mixed in pickles and nutraceuticals pickles are useful for consumers.

Total Flavonoid Contents

The total flavonoid contents were determined by aluminium chloride assay. The values of total flavonoid contents were in the range of (77.12 ± 0.01 to 43.09 ± 0.05 QE mg/g). The maximum total flavonoid contents 77.12 ± 0.01 QE mg/g were present in (P3) while lowest flavonoid contents 43.09 ± 0.05 QE mg/g were observed in (control). The detailed results were discussed in Table 1. The results of research compared with previous literature. The leaf mustard pickle contained total flavonoid contents in range of 4.54 ± 0.03 - 1.96 ± 0.01 QE mg/g. DLMP methanol extracts at 14-day storage indicated highest flavonoid contents 4.54 ± 0.03 QE mg/g while lowest flavonoid contents 1.96 ± 0.01 QE mg/g of DLMP ethanol extracts was at 0-day storage [13]. The motivating information of research was that total flavonoid contents were increased by increasing the nutraceuticals in pickles.

Antidiabetic Activity

The antidiabetic activity was determined by alpha amylase assay and results were expressed by percentage inhibition. The values of inhibition were lies in the range of ($IC_{50} = 25.89 \pm 0.07$ to 11.67 ± 0.06 μ g/mL). The highest inhibition

$IC_{50} = 25.89 \pm 0.07$ μ g/mL was observed by (P3) while lowest inhibition $IC_{50} = 11.67 \pm 0.06$ μ g/mL was indicated by (control). The acrobiose was used as a standard found to ($IC_{50} = 31.26 \pm 0.05$ μ g/mL). The detailed results were shown in Table 2. According to the previous literature the antidiabetic activity of different solvent frictions of *Heliotropium strigosum* were lies in the range of $IC_{50} = 100 \pm 0.15$ to 10.45 ± 0.01 [10]. The informative fact of our research was that if different nutraceuticals were added then antidiabetic activity increased. So, Inhibition was directly related to the concentration of nutraceuticals.

Sensory Evaluation

According to sensory evaluation all pickles were accepted for use and all got score more than 50 out of 100 but P3 pickles which contained more nutraceuticals as compared to other samples were highly appreciated by consumers while P1 pickle was got minimum score from consumers as compared to other pickle samples. The detailed results were discussed in Table 2. According to the pervious findings the sensory evaluation of tomato seed oil was indicated that the tomato seed oil was highly appreciated by consumer if compared with control [14].

Determination of Heavy metals

The heavy metals were determined by wet digestion assay and results were expressed in parts per million (ppm). Total seven heavy metals (Pb, Cd, Zn, Cu, Fe, Co and Ni) was analysed in home made pickles samples qualitatively and quantitatively. The concentration of Lead (Pb), cadmium (Cd), zinc (Zn) copper (Cu) Iron (Fe), cobalt (Co) and nickel (Ni) were (0.23 ± 0.02 to 0.08 ± 0.01 mm), (0.50 ± 0.08 to 0.18 ± 0.03 mm), (27.12 ± 0.01 to 19.09 ± 0.05 mm), (27.84 ± 0.01 to 13.71 ± 0.04 mm), (29.06 ± 0.09 to 18.46 ± 0.01 mm), (23.55 ± 0.03 to 12.77 ± 0.04 mm) and (29.45 ± 0.05 to 18.21 ± 0.01) respectively. The concentration of heavy metals in the pickles were below the permissible limit. The detailed results were discussed in

Table 1: Antioxidant activity, total phenolic contents, total flavonoid contents and antidiabetic activity of nutraceuticals pickles

Pickle samples	Antioxidant activity (% inhibition)	Total phenolic contents (mg GAE/100 g)	Total flavonoid contents (QE mg/g)	Antidiabetic activity IC_{50} (μ g/mL)
P1	$83.23^c \pm 0.07$	$56.12^c \pm 0.04$	$9.31^b \pm 0.03$	$19.00^c \pm 0.09$
P2	$79.34^b \pm 0.01$	$52.40^b \pm 0.09$	$5.98^c \pm 0.08$	$15.29^d \pm 0.02$
P3	$87.56^a \pm 0.02$	$77.12^a \pm 0.01$	$14.67^a \pm 0.06$	$25.89^b \pm 0.07$
Control	$64.11^d \pm 0.01$	$43.09^d \pm 0.05$	$2.31^d \pm 0.08$	$11.67^e \pm 0.06$
Standard	$89.54^a \pm 0.03$	--	--	$31.26^a \pm 0.05$

Standard for antioxidant activity: Ascorbic acid.

Standard for antibacterial activity: Acrobiose

Table 2: Sensory evaluation of nutraceuticals mixed pickles

Pickle Samples	Sensory attributes				
	Odour(25)	Appearance(25)	Colour(25)	Taste(25)	Overall acceptability(25)
P1	$16.55^c \pm 0.03$	$16.76^{bc} \pm 0.04$	$13.76^c \pm 0.01$	$16.34^c \pm 0.02$	$73.44^c \pm 0.01$
P2	$18.12^b \pm 0.02$	$17.00^b \pm 0.01$	$15.12^b \pm 0.09$	$19.63^b \pm 0.08$	$78.56^b \pm 0.04$
P3	$21.67^a \pm 0.07$	$22.76^a \pm 0.02$	$18.92^a \pm 0.02$	$22.76^a \pm 0.01$	$83.65^a \pm 0.02$
Control	$11.56^d \pm 0.01$	$13.76^c \pm 0.06$	$12.89^c \pm 0.04$	$13.75^d \pm 0.02$	$68.12^d \pm 0.01$

Table 3: Concentration of heavy metals in mixed pickles

Pickle Samples	Concentration in ppm						
	Pb	Cd	Zn	Cu	Fe	Co	Ni
P1	0.08 ^d ±0.01	0.50 ^a ±0.08	22.89 ^b ±0.07	19.27 ^c ±0.02	29.06 ^a ±0.09	23.55 ^a ±0.03	26.98 ^b ±0.03
P2	0.40 ^c ±0.03	0.18 ^d ±0.03	27.12 ^a ±0.01	27.84 ^a ±0.01	27.24 ^b ±0.01	18.76 ^b ±0.06	23.78 ^c ±0.03
P3	0.12 ^b ±0.09	0.29 ^c ±0.01	19.09 ^c ±0.05	24.19 ^b ±0.09	21.33 ^c ±0.03	16.98 ^c ±0.09	18.21 ^d ±0.01
Control	0.23 ^a ±0.02	0.42 ^b ±0.04	21.11 ^{bc} ±0.02	13.71 ^d ±0.04	18.46 ^d ±0.01	12.77 ^d ±0.04	29.45 ^a ±0.05

Table 3. According to the previous finding the commercialized pickles purchased from local market of Bangladesh contained lead, zinc and copper below detection limit [2].

CONCLUSION

Our study to the best of our knowledge reported for first time to check the effect of nutraceuticals on antioxidant activity, total phenolic contents, total flavonoid contents, antidiabetic activity, sensory evaluation and heavy metal contents of mixed vegetable pickle. The results of our study showed that the mixed pickle which contained more medicinal plant powders showed excellent antioxidant activity, total phenolic and flavonoid contents, antidiabetic activity and sensory evaluation as compared to other while lowest potential observed in control which was did not contain nutraceuticals. So, our study suggested that the nutraceuticals are useful against different diseases and beneficial for use. The obtained sensory evaluation results were encouraging in terms of value addition of nutraceuticals.

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