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Effects of Synthetic and Natural Extraction Chemicals on Functional Properties, Polyphenol Content and Antioxidant Activity of Soy Protein Isolates Extracted from Full-Fat and Defatted Flours

[1, 2]Moses Vernonxious Madalitso Chamba, [1]Yufei Hua, [1]Quirino Dawa,
[1]Odlon Jakpo and [1]Caimeng Zhang
[1]School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue,
214122 Wuxi, Jiangsu Province, P.R. China
[2]Department of Human Ecology, Domasi College of Education, University of Malawi,
P.O. Box 49 Domasi, Zomba, Malawi

Abstract: The number of consumers preferring natural and organic foods to those that involve synthetic chemicals has recently shown dramatic growth. As such a native isoelectrically precipitated Soy Protein Isolates (SPIs) were prepared from full-fat and defatted flours using amaranth (*Aamaranthus tricolor* L.) lye (pH>12.5) and lemon extract (pH<2.5) as natural food-plant-based chemicals, replacing the conventional synthetic ones (NaOH and HCl, respectively). Functional properties, total polyphenol content and antioxidant activity of the natural SPIs were compared to those of synthetic ones. All the SPI samples qualified to be soy protein isolates with a minimum protein content of 91.21%. The natural SPI showed significant increase in emulsion stability ($p \leq 0.05$). While higher values with narrow margins were shown by the synthetic than the natural SPIs in oil absorption (0.66±0.02, 0.50±0.01%, respectively), emulsion capacity (56.53±0.57, 55.50±0.39%), foam stability (11.33±0.61, 10.40±0.40%). No significant difference was observed in water absorption capacity. The DPPH assay showed increased antioxidant activity in the natural SPI although its total polyphenol content was lower. Thus, SPI with functional properties similar to those of conventional ones can be naturally processed using amaranth ash and lemon extracts as alternative chemicals to addressing the fear of consuming synthetic chemical by health-conscious natural and organic food consumers.

Key words: Functional properties, amaranth ash extract, lemon extract, natural SPI, conventional SPI

INTRODUCTION

The past few years have shown dramatic growth in organic, natural and environmentally friendly or green food market. As many people are becoming more conscious about their health, uncertainty about safety of the highly processed foods, especially in terms of its long term effect as they interact with one another in the body is rising. Studies on food preferences have shown fear of exposure to non-food synthetic chemicals and environmental consciousness as major factors influencing this new food consumer behavior (Dickson-Spillmann *et al*., 2011). Recent years have witnessed an attempt by the agricultural industry to produce food naturally without using synthetic chemicals. Likewise, food manufacturers are also trying to explore new food processing techniques to address the need for safer food and compete for consumer acceptance (Zink, 1997).

Various modern and partially traditional techniques are currently being employed by the food industry to process plant agricultural products such as soybeans, into fat-reduced, protein-enriched compositions for use in food manufacturing. They include solvent extraction and a variety of press-based methods, e.g., extruder, expeller, continuous and cold presses to separate at least a portion of the fat from the remaining plant material. Nevertheless, at some stage strong non-food synthetic chemicals are still used to finalize the process. Some of these chemicals are not natural or plant based and cannot be used to produce certified organic food products under United States Department of Agriculture (USDA) guidelines for organic food labeling (Gold, 2007). As such, a lot of improvements are yet to be made on highly processed foods.

Soy Protein Isolate (SPI), a soybean derivative protein powder is one of the most important products in food processing as well as many other industrial uses. Its

Corresponding Author: Moses Vernonxious Madalitso Chamba, School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, 214122 Wuxi, Jiangsu Province, P.R. China

preference is attributed to its ability to enhance nutritional (especially protein) and functional qualities of food products to which it is used as an ingredient (Kinsella, 1979; Mariotti *et al*., 1999; L'Hocine *et al*., 2006). SPI has been added to baked foods, breakfast cereals and meat products among others. It can also be made into a nutritious drink once added to water. A soybean product qualifies to be called a protein isolate only when it contains at least 90% crude protein (N×6.25) on dry basis (Codex Alimentarius Commission, 1996). To achieve this purity, conventional technique of processing SPI involves use of synthetic chemicals such as n-hexane, ethanol, sodium hydroxide (NaOH) and Hydrochloric acid (HCl) (Kinsella, 1979). These chemicals have well known risks if handled without adequate without taking precautions and their health hazard cases, especially with direct contact in adequate doses have been widely reported. Although, some these chemicals are removed during the process having some small residues in the final product cannot be exclusively denied. Furthermore, a health conscious natural food consumer cannot be convinced that foods processed using these chemicals are safe to eat. Dickson-Spillmann *et al*. (2011) reported that laypeople perceive chemicals as either safe or hazardous and think that even minor doses of chemicals are likely to cause harm. To such people, synthetic equals dangerous. The challenge lies therefore on identifying natural and food-based reagents and chemicals that would produce similar or improved results as the synthetic ones. At this time when consumers are increasingly becoming skeptical about consuming food processed using synthetic chemicals, more exploration on the traditional chemistry may be considered a favorable option just as it is in traditional medicine.

Amaranth ash solution has been used traditionally for food preparation as an alternative to bicarbonate of soda (alkaline). In countries like Malawi, amaranth (*Amaranth hybridus*) plant is the most preferred source of ash for food alkali due to its pH strength and safety for consumption. However, information on the same can scarcely be found. Lemon extract as citric acid is a well known food acid but rarely mentioned in soybean products studies.

In the earlier study, it was observed that soy protein isolate with acceptable yield, composition and nutritive quality can be produced using purely natural, food-plant-based chemicals such as amaranth ash and lemon extracts as alternative to NaOH and HCl, respectively. The aim of this study was therefore; to examine the functional properties (water and oil absorption capacity, emulsifying capacity and stability, foam formation and stability, gelation), Total Polyphenol Content (TPC) and antioxidant activity of the SPIs prepared using these natural chemicals with comparison to those in which the conventional synthetic chemicals were involved.

MATERIALS AND METHODS

Edible green and purple colored amaranth (*Amaranthus tricolor* L.) plants were obtained from local farmers in the outskirt of Wuxi City, Jiangsu Province, China. Ripe lemon (*Citrus limon*) fruits were purchased from a local market. Soybean grains were supplied by Suzhou Golden Village Food Co., Ltd. Jiangsu Province, China. The soybeans were sorted, cleaned (to remove unrelated materials), dehulled then ground into native full fat flour, fine enough to pass through an 80 mesh sieve. On dry basis, the flour had 35% protein (N×6.25), 24% lipid, 3% ash, 7% moisture and Protein Dispersibility Index (PDI) of 72%. One part of the flour was treated with n-hexane and 95% alcohol to remove fat and soluble sugars. Amaranth ash extract (pH≥12.50) was prepared by filtering distilled water through burnt amaranth plant ash at the ash to water ratio of 1:5 (w/v) after optimization. Lemon extract (pH<2.20) was prepared by squeezing the lemons then vacuum filtering the extract to remove the pulp. DPPH (2,2-diphenyl-1-picryl hydrazyl) was purchased from Sigma, Shanghai. All other reagents and chemicals were of analytical grade. Deionized or distilled water was used in all laboratory procedures and sample preparations.

Preparation of soy protein isolate: Four SPI samples namely; Synthetic Chemical Full Fat flour (SCFF), Synthetic Chemical Defatted Flour (SCDF), Natural Chemical Full Fat flour (NCFF) and Natural Chemical Defatted Flour (NCDF) were prepared as described by Li *et al*. (2007) with modifications. Appropriate flour (full fat or defatted) was suspended in distilled water at the flour to water ratio of 1:10 (w/v). The pH of the suspension was adjusted to and maintained at 7.0 with either amaranth extract or NaOH while stirred for 1 h at ambient temperature 22±2°C. After centrifugation, at 10000×g and 4°C for 30 min (for SCFF, SCDF and NCDF SPIs), the supernatant was recovered. Soy protein was precipitated by adjusting pH to 4.5 with lemon extract or HCl then centrifuged at the above conditions. The precipitate was washed and centrifuged twice before suspended again in distilled water and neutralized to pH 7.0 with either amaranth extract or NaOH. All resolubilised SPI samples were freeze-dried, sealed in polythene bags and stored at 4°C until further analysis.

Proximate analyses: Proximate analyses of the four samples were conducted as follows: crude protein was determined by Micro-Kjeldahl Method with the common conversion factor of N×6.25 (AOAC, 2000). Crude oil was extracted the traditional Soxhlet extraction apparatus and determined according to AOAC Official Method (AOAC, 2000). Total ash was analyzed using the conventional method by dry-ashing in muffle furnace at 550°C. Moisture content was calculated by drying weighed samples for 3 h in an oven at 120°C (Krober, 1966).

Functional properties

Oil and water absorption capacities: Oil Absorption Capacity (OAC) and Water Absorption Capacity (WAC) were assessed in triplicate using the procedure described by Lin *et al.* (1974). A protein sample (0.3 g) was mixed with soy oil or distilled water (1.5 mL) in a pre-weighed 15 mL graduated centrifuge tubes and centrifuged for 30 min at 4000 rpm. The supernatant was discarded and the tubes were reweighed. The OAC and WAC (%) were expressed using the following equation:

$$\frac{FAC}{WAC}(\%) = \frac{(W_0+W_1)}{W_0}\times 100$$

Where:

W_0 = The initial weight of the sample

W_1 = The weight of sample plus the absorbed liquid

Foaming capacity and stability: Foams were prepared according to Sathe *et al.* (1982) with minor modifications. Briefly, 2 g of each SPI sample was solubilised in 200 mL of distilled water to make a 1% solution. Aliquots of 50 mL were poured into graduated 250 mL, 3 cm diameter cylinder and whipped by high shear stepless speed homogenizer (model: FA 25, Fluco Equipment Co., Ltd. Shanghai, China) at 10000 rpm, ambient temperature (20±2°C) for 3 min. A smooth round end glass rod was used to level the foam for easy reading. Foam capacity was calculated as percentage rise in total volume at 0 times. Stability was determined as a factor of foam drainage recorded after 30 min:

$$\text{Foaming capacity}(\%) = \frac{(V_1-V_0)}{V_0}\times 100$$

$$\text{Foam stability}(\%) = \frac{V_2}{V_0}\times 100$$

Where:

V_0 = Initial sample volume before whipping

V_1 = Total sample volume after whipping at time 0

V_2 = The water volume still trapped in foam after 30 min

Emulsifying capacity and stability: For emulsion properties, the method described by Yatsumatsu *et al.* (1972) was used with minor modification. Protein isolate solution (1%, w/v) was prepared in 30 mL of distilled water in a beaker, magnetically stirred for 1 h to solubilise the protein. Then, 30 mL of soy oil was added. The mixture was homogenized by a rod homogeniser (model: FA 25, Fluco equipment Co., Ltd. Shanghai, China) for 0.5 min at 10000 rpm. Triplicate aliquots (12 mL each) of the freshly prepared emulsion was poured into calibrated centrifuge tubes and centrifuged at 2500 rpm for 15 min. The ratio of the height of water layer to the total liquid layer was used to calculate Emulsifying Activity (EA). The emulsion was then heated at 70°C for 30 min in a water bath, followed by 15 min of cooling under running tap water and centrifugation at the same conditions. The stability was worked out using the height of the two layers. Results were calculated as follows:

$$\frac{EA}{ES}(\%) = \frac{(V_0-V_1)}{V_0}\times 100$$

Where:

V_0 = The initial volume of the total liquid in the centrifuge tube

V_1 = The volume of water layer

Least gelation concentration: The least gelation concentration was determined by the method of Coffmann and Garciaj (1977). Test tubes containing 5 mL protein suspensions of 5, 6, 7, 8 up to 15% (w/v) in distilled water were heated for 1 h in boiling water, followed by cooling in ice bath and further cooling for 2 h at 4°C. The results were reported as liquefied (-), gluey (±) and gel (+). The least gelation concentration was the minimum concentration at which the sample did not fall down or slip when the test tube was inverted.

Determination of total polyphenol content and antioxidant activities

Total Polyphenol Contents (TPC): To extract polyphenols, a sample (1 g) was suspended in 25 mL methanol-water 80:20 v/v acidified with 0.1% HCl and magnetically stirred for 1 h at room temperature. Later, the mixture was centrifuged at 1800×g for 15 min, the methanol was decanted and the residue extracted again with 25 mL of fresh aqueous methanol. After centrifugation at same conditions, the two extracts were combined (Akowuah *et al.*, 2005; Mujica *et al.*, 2009).

Total Phenols Content (TPC) of the SPIs was determined by Folin-Ciocalteau phenol reagent as described by Skerget *et al.* (2005) with some modification using garlic acid monohydrate (0-60 μg L^{-1}, N = 7) as a

standard. To 0.5 mL of appropriately diluted sample, 2.5 mL of Folin-Ciocalteu reagent was added. After that (within time interval from 0.5-8 min), 2 mL of Na_2CO_3 (7.5%) was added. The mixture was incubated for 5 min at 50°C and then cooled. For a control sample, 0.5 mL of distilled water instead of sample was used. The absorbance was measured at 760 nm by UV/Vis spectrophotometer (UV-2450, Shimadzu Corporation, Japan). The content of total phenols was expressed as garlic acid equivalents (GAE) in μg/g SPI. All analyses were run in triplicate and mean values were calculated. The calibration equation of garlic acid was:

$$y = 0.05997x-0.03535,\ R^2 = 0.9997$$

DPPH free radical-scavenging assay: DPPH free radical-scavenging assay was determined according to the method of Brand-Williams *et al.* (1995) with modification. A 2 g of each SPI sample was extracted twice (2 h each time) with 20 mL aqueous methanol (80% containing 0.1% HCl) centrifuged at 2500 rpm for 15 min and supernatant combined. Then, a series of working solutions of different concentrations (0.5, 1, 2, 3, 4 and 5 mg mL^{-1}) were prepared. A 3.5 mL of methanolic DPPH (2,2-diphenyl-1-picryl hydrazyl, 0.06 mM) solution was added to 1.5 mL diluted sample or methanol (for control) and vortexed. The mixture was incubated for 30 min at room temperature in the dark. The absorbance was read at 517 nm by UV-Vis spectrophotometer (UV-2450, Shimadzu Corporation, Japan) against the DPPH solvent as blank. The inhibition percentage of free radical by sample was calculated using the following formula and the results presented as a comparative graph.

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where:

A_0 = The absorbance of the control (blank, without sample)

A_1 = The absorbance in the presence of the sample

Statistical analysis: One way Analysis of Variance (ANOVA) with Duncan"s multiple range test was conducted using a SAS program (Version 8.1, SAS Institute Inc., Cary, NC, USA) to asses significance of differences ($p<0.05$) among means of triplicate sample runs.

RESULTS AND DISCUSSION

Proximate analysis: Values for proximate analysis are presented in Table 1. The SPIs prepared from either full-fat soybean flour or natural chemicals (ash and lemon extracts) showed slightly lower protein compositions as compared to the one from both defatted flour and conventional synthetic chemicals (NaOH and HCl) respectively.

A soy product is recognized as an isolate only if it contains not <90% protein (Preeti *et al.*, 2008). Thus, all samples including the NCFF, qualified to be SPIs. The rest of the compositions were within the recommended ranges of a good quality SPI according CODEX STAN 175-1989 general standards (Codex Alimentarius Commission, 1996). The lower protein composition observed by NCFF may be attributed to high fat in the starting material (full-fat flour), ash composition in the solvent (ash extract) and remaining pulp in the acid (lemon extract).

Functional properties

Oil and water absorption capacities: Results of the oil and water absorption capacities are presented in Table 2. The WAC was significantly higher in SCFF SPI (0.62±0.05 mL g^{-1}) followed by SCDF and NCFF (0.55±0.04, 0.55±0.03 mL g^{-1}, respectively). NCDF SPI showed the lowest value. In their study, Fleming *et al.* (1974) and Deshpande *et al.* (1982) attributed improved water absorption capacity to the increase in protein content of the sample. Similarly, higher WAC has been observed in protein isolates in comparison with protein concentrates (Fleming *et al.*, 1974; Lin *et al.*, 1974; Wang and Kinsella, 1976; Hutton and Campbell, 1977) probably indicating the relationship between water absorption and protein content. However, the findings of this study did not fully agree with the protein content of the samples (Table 1). Inasmuch as protein content between the two main samples (SCDF and NCFF) was substantially different, there was no difference in water absorption indicating that other factors might had played a role. Lin *et al.* (1974) observed that sunflower

Table 1: Proximate analyses of the SPI samples

Contents	NCFF	NCDF	SCFF	SCDF
Protein (%)	91.21	93.64	92.12	98.45
Lipid	0.71	<0.05	0.65	<0.05
Ash	5.42	5.91	3.83	2.08
Moisture	3.38	3.24	2.49	2.84

Table 2: Water and oil absorption, emulsion and foaming properties of the traditional and conventional soy protein isolates

Samples	Water absorption (mL g^{-1})	Oil absorption (mL g^{-1})	Emulsion capacity (%)	Emulsion stability (%)	Forming capacity (%)	Form stability (%)
SCFF	0.62±0.05^{a}	0.59±0.01^{b}	55.83±0.66^{b}	52.30±1.64^{b}	127.33±3.06^{c}	9.80±0.70^{b}
SCDF	0.55±0.04^{a}	0.66±0.02^{a}	56.53±0.57^{b}	54.51±0.92^{b}	231.33±4.16^{a}	11.33±0.61^{a}
NCFF	0.55±0.03^{a}	0.50±0.01^{c}	55.50±0.39^{c}	54.71±1.00^{a}	110.00±5.29^{d}	10.40±0.40^{b}
NCDF	0.32±0.05^{b}	0.37±0.01^{d}	57.47±0.33^{a}	53.43±0.89^{b}	217.33±7.02^{b}	9.60±0.40

$^{a-d}$Values with different superscript letters along the same column indicate significant difference ($p\leq0.05$) among means of triplicates tests

products had lower water absorptions than did soy products although their protein contents were similar. These finding indicate that water absorption of a particular sample need not always be parallel to the protein content other food components may have an influence.

The Oil Absorption Capacity (OAC) differed significantly in all samples (SCDF>SCFF>NCFF>NCDF) as shown in Table 2. The differences were within a range between 0.37±0.01 mL g^{-1} (NCDF) and 0.66±0.02 mL g^{-1} (SCDF) for all samples and from 0.50±0.01 mL g^{-1} (NCFF) to 0.66±0.02 mL g^{-1} (SCDF) between the two main samples (traditional and conventional SPIs, NCFF and SCDF, respectively). Oil absorption values seemed to fall within the same ranges with those of water absorption. Oil absorption is mainly attributed to the physical entrapment of oil and is related to the number of nonpolar side chains on proteins that bind hydrocarbon chains of fat (Lin *et al*., 1974; Kinsella, 1979). The increased absorption in SCDF is then associated with increased nonpolar side chains in the sample due to its higher protein content.

Foaming capacity and stability: Volume increase of the 1% aqueous dispersion of all the SPI samples ranged from 110.00±5.29% (NCFF) to 231.33±4.16% (SCDF) after whipping as shown in Table 2. The specific volumes of the foams were determines as indicators of air uptake during whipping. The lower air uptake of the SPI prepared with the natural chemicals and full-fat flour was associated with decrease in its foam volume. Specific volume of a foam (volume per unit mass) for a given set of conditions is an indication of air uptake only and is not necessarily a measure of foam quality and stability (Deshpande *et al*., 1982).

The foam stability was determined as a factor of foam drainage after 30 min, other than the volume maintained by of the foam. Observation has shown that foam has a capability of maintaining their volume by sticking to the side of the container even if it is weak. Usually, the amount of liquid retained in the foam is a better indication of foam strength or quality. Although, the total volumes just after whipping at ambient temperature (16±1°C) differed significantly ($p<0.05$) in all samples (Table 2), their stabilities did not differ except in SCDF where it was higher. Thus, the stability of the foam may not only be attributed to protein concentration alone as indicated by Deshpande *et al*. (1982) but also to other components to which the liquid gets entrapped. The effects of pH on both foam capacity and stability have been reported (Li *et al*., 2007; Sekul *et al*., 1978; Canella *et al*., 1979; Cherry and McWatters, 1981). However, the fact that all samples were treated under the same pH condition, it may not be associated with the differences.

Emulsifying capacity and stability: The Emulsion Capacity (EC) and Emulsion Stability (ES) of all the SPI samples are given in Table 2. Comparison of the main natural and conventional samples (NCFF and SCDF) showed a significant decrease ($p<0.05$) of EC with a very narrow margin (1.86%) in NCFF than SCDF SPIs. The highest EC was observed in one of the intermediate samples (NCDF). However, the natural SPI demonstrated the most significantly ($p<0.05$) stable emulsion than the rest of the samples whose ES did not differ. At the same concentration, the ES of all the samples seemed lower that those reported by others. This may be attributed to the method used to prepare the emulsions. It has been reported that in emulsions prepared using the shearing force method, the stabilization property of heated soy protein isolate decreased to a lower level when it was compared to those prepared by other methods (Zayas, 1997). Stabilization of the emulsions by protein polymers is relevant in almost all food applications. Protein polymers have two effects: they can gel in the continuous phase at high protein concentrations through protein cross-linkings and they provide strong repulsive forces when absorbed at the oil/water interface (Ma and Barbosa-Canovas, 1995).

Least gelation concentration: Gelation may be defined as a protein aggregation phenomenon in which polymers-polymer and polymer-solvent interactions and attractive and repulsion forces are so balanced that a tertiary network or matrix is formed (Schmidt, 1981). Such matrix has a capability of trapping and immobilising large amount of water. Protein concentration, other protein components, in a complex food system, non protein components, heat treatment conditions, pH and ionic and reducing agents are some of the factors that affect gelation (Schmidt, 1981). Comparison of the main natural and conventional SPIs indicated similarity with the findings observed in the foaming property where higher values were observed in the SPIs prepared from defatted flour than full-fat flour. The greatest value was recorded in NCFF (14%) and the lowest in SCDF and NCDF (both 10%) as presented in Table 3. This was not surprising as it related to the protein content of the samples which was higher in those prepared from defatted soy flour and was least in NCFF SPI.

Depending on food product to which a protein isolate is used as an ingredient, both high and low gelation properties are desirable. These results showed that the natural chemical prepared SPI had low gelation property compared to the conventional one. By using non-defatted flour and lemon and ash extracts which were not thoroughly purified, NCFF contained a complex of

Table 3: Least gelation concentration of SPI samples[a]

Concentration (%)	SCFF	SCDF	NCFF	NCDF
5	(-)	(-)	(-)	(-)
6	(-)	(-)	(-)	(-)
7	(-)	(-)	(-)	(-)
8	(±)	(-)	(-)	(-)
9	(±)	(±)	(-)	(±)
10	(±)	(+)	(±)	(+)
11	(+)	(+)	(±)	(+)
12	(+)	(+)	(±)	(+)
13	(+)	(+)	(±)	(+)
14	(+)	(+)	(+)	(+)
15	(+)	(+)	(+)	(+)
LGC	11	10	14	10

[a]Gelation levels: (-) liquefied, (±) gluey and (+) gel; the values are the mean of three replicates

of particles and compound such as carbohydrates and metal elements that might interfere with formation of continuous network of the protein molecules to form a gel. A higher protein concentration thus may be required to overcome their interference with gel formation. Schmidt *et al.* (1978) reported that dialysis of whey protein concentrate system improved its gelation property. Nevertheless, more information is required on the effects of components other than the SPI itself on protein gelation properties before predictions regarding the gelation behavior of this natural SPI in a complex food system can be made.

Determination of total polyphenol content and antioxidant activities

Total Polyphenol Contents (TPC): The results of the Total Polyphenol Content (TPC) showed a wide difference between samples prepared using different chemicals but no significant differences were observed in SPIs prepared from the same chemicals. SCFF and SCDF contained higher TPC (23.80±1.93 and 22.69±2.67 μgGAE g^{-1}, respectively) than both NCFF (11.02±1.27 μgGAE g^{-1}) and NCDF (9.40±1.33 μgGAE g^{-1}). Consequently, it was expected that the conventional chemically prepared samples would demonstrate increased antioxidant activity than their natural counterparts. Nevertheless, many compounds such as vitamin C which is contained in lemons among other sources are also responsible for the antioxidant activity. This had to be verified by conducting an antioxidant activity determination.

DPPH free radical-scavenging assay: DPPH free radical-scavenging assay using either 2,2-diphenyl-1-picryl hydrazyl or 1,1-diphenyl-2-picryl hydrazyl radical is one of the most common methods for evaluating antioxidant activity of food samples. It is simple, rapid, generally accurate and inexpensive (Prakash *et al.*, 2001). Additionally, different researchers have used different ways of reporting DPPH antioxidant assay results either

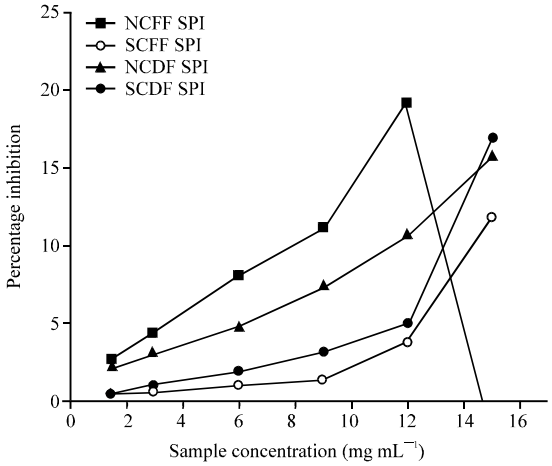

Fig. 1: Antioxidant activity by 2,2-diphenyl-1-picryl hydrazyl (DPPH, 0.06 mM) assay

graphically or by calculating IC_{50}, a value at which 50% of the used DPPH has reacted with the sample (Marinova and Batchvarov, 2011). In this study, the graphical presentation was preferred to show the exact absorption behavior of the samples. As shown in Fig. 1, the DPPH assay results of this study showed that the SPI prepared using the traditional chemical demonstrated increased antioxidant activity (with NCFF having the highest) than those using conventional chemicals. This increase may be attributed to the presence of lemon extract which is one of the well known sources of vitamin C, a natural antioxidant. If this is really the case then the activity may vary depending on the vitamin C composition of lemons used.

It was also observed that at a certain concentration, the Natural SPI (NCFF) showed serious reduction in absorbance regardless of repetition of the test for a number of times. There is possibility that due to the impurity of the natural chemicals, the sample contained certain components such as carotenoids which are normally present in the lemons that at increased concentration substantially interfered with coloration of the assay (Nomura *et al.*, 1997) but not necessarily the antioxidant activity of the sample.

CONCLUSION

The findings of this study suggest that instead of the conventional synthetic NaOH and HCl, traditional chemicals such as amaranth plant ash and lemon extracts can be used to prepare SPI, not only with desirable functional properties similar to those of the conventional ones but also with increased antioxidant activity. Basic as it may seem, this method of SPI processing may have

effective outcomes of improving livelihood of indigenous people in terms of food supplementation, in addition to providing a possible alternative to the synthetic chemicals which are increasingly feared by the health-conscious food consumers. Much as the functional properties of soy protein isolates have been associated with the protein content of the product, the findings of this study also suggest that functional properties of SPI cannot apparently be attributes to the protein level but also to its interaction with other components such as ash particles, carbohydrates and lipids.

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