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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF SPICY SAUERKRAUT FERMENTED WITH REDUCED SALT

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ABSTRACT

Sauerkraut fermentation is usually carried out in the presence of high sodium chloride amounts. However, nowadays consumers who prefer to consume a healthy product would opt for a low sodium content food with additional health benefits. Therefore, in the present study, sauerkraut was fermented with the addition of 0.5 % garlic, 0.1 % pepper and of 1.5 % non-iodized sodium chloride. The phytochemical and antioxidant activity of reduced salt spicy sauerkraut was evaluated. Results indicate that the pH of sauerkraut brine ranged between 5.0 to 3.57 over a period of 28 days fermentation. The total acidity expressed as percent lactic acid in sauerkraut ranged between 0.05 to 0.22 showing an increasing in the lactic acid over a period of fermentation. Nutrients like protein, copper, zinc and vitamin K is found to be higher in reduced salt spicy sauerkraut compared to that of traditional sauerkraut. Preliminary phytochemical screening of reduced salt spicy sauerkraut indicates the presence of carbohydrate, flavanoids, quinines, terpenoids, and phenol. Total Polyphenol Content (TPC) of reduced salt spicy sauerkraut was found to be high containing 36.7 mg GAE/100 ml. Spicy Sauerkraut could be a more healthy option for maintaining optimal nutrition goals.

INTRODUCTION

Cabbage (*Brassica oleracea* L.) is one of the most popular winter vegetables grown in India. It is cultivated in 0.245 M ha with the total production of 5.617 M mt and average productivity of 22.9 mt/ha. The major cabbage producing states are Uttar Pradesh, Orissa, Bihar, Assam, West Bengal, Maharashtra and Karnataka. Among these states West Bengal contributes 1.929 M mt of cabbage from 65,000 ha area with an average productivity of 29.6 mt/ha. Cabbage is used as salad, boiled vegetable and dehydrated vegetable as well as in cooked curries and pickles. Fermented cabbage, i.e., sauerkraut, was introduced in the United States by immigrants from Germany and other European countries. Sauerkraut fermentation is spontaneous and relies on a very small population of lactic acid bacteria (LAB), which are naturally present on fresh vegetables, for preservation. It is known that a succession of various LAB species and their metabolic activities are responsible for the quality and safety of these products. NaCl is a widely used chemical in food processing which affects sensory characteristics and safety; in fact, its presence is frequently essential for the proper preservation of the products. Because the intake of high contents of sodium is linked to adverse effects on human health, consumers demand foods with low-sodium content. A 1st step to reduce the use of salt would imply the proper application of this compound, reducing its levels to those technologically necessary. In addition, different chloride salts have been evaluated as replacers for NaCl, but KCl, CaCl2, and ZnCl2 show the most promising perspectives of use. However, prior to any food reformulation, there is a need for exhaustive research before its application at industrial level. Salt reduction may lead to an increased risk in the survival/ growth of pathogens and may also alter food flavor and cause economic losses (Bautista-Gallego *et al*., 2013).

One of the ways to reduce sodium content in food products is by replacing sodium chloride with other ingredients. Potassium chloride as a partial replacement for sodium chloride. KCl has ionic force properties similar to those of sodium chloride, but its addition can damage its sensory quality of the food by reducing the salty taste resulting in a bitter, metallic, and astringent taste. Hence, using herbs and spice blends is a promising alternative to improve the quality of food products when the partial replacement of sodium chloride occurs. Since they can give a spicy flavor and different aroma. Moreover, some herbs and spices have a proved antioxidant and antimicrobial activity. Commonly applied spices act as flavorings agents. Furthermore, some spices contain powerful antioxidants that can extend the shelf life food products. The two most potent spices are garlic (*Allium sativum*), in which several antimicrobial components are present; the principal

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active substance was identified as allicin and *Piper nigrum* L. is considered the king of spices throughout the world due to its pungent principle piperine. Medicinal properties of garlic (Allium sativum) have been widely known and used since ancient times till the present. Garlic enhances immune functions and has antibacterial, antifungal and antivirus activities. It is known to prevent platelet aggregation, and to have hypotensive and cholesterol- and triglyceride-lowering properties (Iciek *et al*., 2009). The biological role of this spice is explained in different experiments that peppercorn and secondary metabolites of piper nigrum can be used as antiapoptotic, antibacterial, anti-colon toxin, antidepressant, antifungal, antidiarrhoeal, anti-inflammatory, antimutagenic, anti-metastatic activity, antioxidative, antiriyretic, antispasmodic, antispermatogenic, antitumor, antithyroid, ciprofloxacin potentiator, cold extremities, gastric ailments, hepatoprotective, insecticidal activity, intermittent fever and larvisidal activity (Nisar *et al*. 2012). The addition of garlic and pepper during the reduced salt fermentation process might reduce the formation of moulds and might improve the antimicrobial property of sauerkraut. Therefore, the objective of this study was to evaluate the influence of the addition of garlic and pepper with reduced NaCl during fermentation of cabbage on the antioxidant activity of sauerkraut.

MATERIALS AND METHODS

The experimental design was carried out in the following phases

Phase I: Collection of cabbage and formulation of sauerkraut

For the preparation of sauerkraut, the cabbages (*Brassica oleracea*) were obtained from the local market of Chennai. The spotted and defective cabbage heads were trimed off. Cabbage was prepared by removing the outer leaves and core. The cabbage was shredde with a sterile knife into 2 mm x 10 mm. The shredded cabbages were weighed (1 kg) followed by the addition 0.5 % garlic, 0.1 % pepper and of 1.5 % non-iodized sodium chloride. The shredded cabbage, garlic, pepper and the salt were placed in alternating layers in a sterile glass jar. Pressure was applicd to gently squeeze out the brine. The glass jar was covered with sterile lids and incubated at 21 – 24 C for 28 days for the natural fermentation of the cabbage.

Phase II: Evaluation of chemical changes througth measurement of pH

Chemical changes were monitored by measurement of pH Brine samples were taken aseptically through a septum in the lid of each jar after 0, 1, 7, 14 and 28 day of fermentation.

Phase III: Evaluation of Selected Chemical Constituents

Quantitative analysis of carbohydrate, protein, fiber, zinc, copper, potassium and vitamin K and titratable acidity was evaluated using standard AOAC procedures.

Phase IV: Quanlitative phytochemical analysis

The phytochemical tests was carried out using standard methods of analysis of carbohydrates, tannins, saponins, flavanoids, alkaloids, quinines, glycosides, cardiac-glycosides, terpenoids, triterpenoids, coumarins, steroids, phytosteroids, phlobatanins and anthroquinones.

Test for Carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

Test for Tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for Flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for Alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for Quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for Glycosides

To 2ml of plant extract, 3ml of choloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for Cardiac Glycosides

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

Test for Terpenoids

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

Test for Phenols

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

Test for Coumarins

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

Tests for Steroids and Phytosteroids

To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Tests for Phlobatannins

To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

Tests for Anthraquinones

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

Phase V: Quantitative phytochemical analysis

Determination of total phenol content

The amount of total phenol content of sample was determined by Folin-Ciocalteu's reagent method (Mc Donald *et al*., 2001). The various concentrations of the extract were made up with 900µl of distilled water and 0.5 ml of Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 minutes. Then, 4 ml of saturated sodium carbonate solution (0.7 N) was added and further incubated for 30 min at room temperature and the absorbance was measured at 765 nm using a digital spectrophotometer, against a blank sample. The calibration curve was made by preparing gallic acid (100 to 300 µg ml-1) solution in distilled water. Total phenol content is expressed in terms of Gallic acid equivalent (mg g-1 of extracted compounds).

RESULTS AND DISCUSSION

Evaluation of chemical changes througth measurement of pH

The pH of the brine from sauerkraut at different stages of fermentation was determined by pH meter. The pH of sauerkraut brine ranged between 5.0 to 3.57 over a period of 28 days fermentation. The total acidity expressed as percent lactic acid in sauerkraut ranged between 0.05 to 0.22 showing an increasing in the lactic acid over a period of fermentation. Literature suggest that the final acidity of sauerkraut should approximately lie between 1.6% - 1.8% during the fermentation period. Higher pH enhances the shelf life stability of the product. Increase in lactic acid also indicates the increase LAB counts in sauerkraut fermentation. LAB requires complete anaerobic state with low pH and elevated salt content. However, the increase in lactic acid content signifies that LAB counts has increase even in a reduced salt concentration. This may be attributed to the presence of spices. Literature indicates that lactic acid production is stimulated by the addition of pepper. The degree of stimulation of lactic acid production was related to the manganese content of the spices. Pepper has a relative high manganese content (Verluyten *et al*., 2004).

Table 1. pH analysis of Sauerkraut during fermentation

Fermentation time (in days)	pH measurement	Titratable Acidity
Day 0	5.0	0.05
Day 1	4.9	0.07
Day 7	4.36	0.10
Day 14	3.71	0.18
Day 28	3.57	0.22

Evaluation of Selected Chemical Constituents

Quantitative analysis of carbohydrate, protein, fiber, zinc, copper, potassium and vitamin K and titratable acidity was evaluated using standard AOAC procedures.

Table 2. Chemical constituents of spicy sauerkraut versus traditional sauerkraut (per 100g)

Principle	Reduced salt spicy sauerkraut	RDA %	Traditional Sauerkraut	RDA %
Calories (kcal)	14	-	22	-
Carbohydrate (g)	2.91	0.5	5	1
Protein (g)	1.35	2.3	1	1.6
Fat (g)	0	0	0	0
Fibre (g)	3	10	3	10
Copper (mg)	0.34	15	0.1	5
Zinc (mg)	0.66	5.5	0.2	1.6
Potassium (mg)	133	3.6	170	4.5
Sodium (mg)	168	8.0	308	14.7
Vitamin K (µg)	89	161	13	23.6
Titrable acidity (Lactic acid %)	0.224	-	0.045	-
RDA given by ICMR (2009)				

Table indicates that the reduced salt spicy sauerkraut has better nutritional profile compared to that of traditional sauerkraut. Nutrients like protein, copper, zinc and vitamin K is found to be higher in reduced salt spicy sauerkraut. Notably, Vitamin K (89 µg) in reduced salt spicy sauerkraut meets 161 percent RDA, while traditional sauerkraut meets only 23.6 percent RDA. In food, vitamin K1 is bound to the chloroplast membrane of leafy green vegetables. Vitamin K is a fat-soluble vitamin that physiologically acts as a cofactor for the posttranslational γ-carboxylation of vitamin K–dependent proteins. Apart from this well-known physiologic function, evidence from in vitro and in vivo studies indicates that vitamin K exerts inhibitory effects on cell growth in several cancer cell lines. Vitamin K catalyses the carboxylation of a number of protein factors involved in blood clotting including prothrombin, forming the calcium binding sites on glutamyl side chains in the protein. Recent studies revealed that vitamin K2 contributes to both bone and cardiovascular health (Sato *et al*., 2012).

Preliminary qualitative analysis of phytochemical presentin in spicy sauerkraut

Plants consist of a number of biologically active ingredients therefore they are used for the treatment of a large number of infectious diseases. These biologically active ingredients are alkaloids, flavonoids, steroids, glycosides, Terpenes, tannins and phenolic compounds (Mohammad Nasir *et al*., 2011). The phytochemical tests was carried out using standard methods of analysis of carbohydrates, tannins, saponins, flavanoids, alkaloids, quinines, glycosides, cardiac-glycosides, terpenoids, triterpenoids, coumarins, steroids, phytosteroids, phlobatanins and anthroquinones. Preliminary Phytochemical screening of reduced salt spicy sauerkraut is presented in Table 3.

Table 3. Qualitative phytochemical analysis

Phyto constituents	Reduced salt spicy sauerkraut extact
Carbohydrate	+
Tannin	-
Saponins	-
Flavanoids	+
Alkaloids	-
Quinines	+
Glycosides	-
Cardiac glycosides	-
Terpenoides	+
Phenol	+
Coumarins	-
sterols and phytosterols	-
Phlobatannins	-
Anthoquinones	-

Preliminary phytochemical screening of reduced salt spicy sauerkraut indicates the presence of carbohydrate, flavanoids, quinines, terpenoids, and phenol. Neelufar *et al*. (2012) in their study has indicated that the red cabbage (Brassica oleracea var. capitata f. rubra) has phytochemicals like carbohydrates, proteins, glycosides, flavonoids, phenolic compounds and proteins. The fermentation of cabbage has been shown to enhance its protective activities. The glucosinolatemoieties in sauerkraut induces the body's antioxidant enzymatic activity and the flavonoid components confer protection to blood vessels from oxidative damage (Felix *et al*., 2014). Some studies have been conducted to quantify the phenolic compounds, carotenoids, vitamin C, and antioxidant potential.

The mechanism of chemo preventive action of cruciferous vegetables is still not fully clarified, however many animal and human intervention studies suggest that the substances present in these plants, especially glucosinolates (GLS) and products of their decomposition, are able to modulate activity of phase I and II enzymes. GLS degradation products are believed to act as ant carcinogens by decreasing carcinogen activation through the inhibition of phase I enzymes, while increasing detoxification by induction of the phase II enzymes that affect xenobiotic transformations. These compounds were also shown to inhibit tumor cell growth and to stimulate apoptosis (Kusznierewicz *et al*., 2008). Flavonoid derivatives with a lower molecular mass (di- and triglycosides) and aglycones of flavonoids and hydroxycinnamic acids were detected in fermented cabbages compared to the main compounds detected in nonfermented cabbages (tri- and tetraglycosides of flavonoids and hydroxycinnamic acid derivatives of malic acid, glycoside, and quinic acid). During the fermentation process, contents of flavonoid derivatives and some hydroxycinnamic acid derivatives were found to decrease. Some marginal losses of polyphenols were observed even in the kneading step of the plant material prior to the fermentation procedure. The antioxidative potential of fermented cabbages was much higher compared to that of nonfermented cabbages in the TEAC assay, but not observable in the DPPH assay. The increase of the antioxidative potential detected in the TEAC assay was attributed to the qualitative changes of polyphenols (Britta *et al*., 2008)

Determination of total phenol content

The protective effects against chronic diseases could also depend on the antioxidant activity of compounds present in cruciferous vegetables. Although some studies have been conducted to determine the antioxidant activity, the content of polyphenols and flavonoids as well as other antioxidants in white cabbage, polyphenol content of sauerkraut has not been studied. Therefore the present study evaluates the total phenolic content of reduced salt spicy sauerkraut.

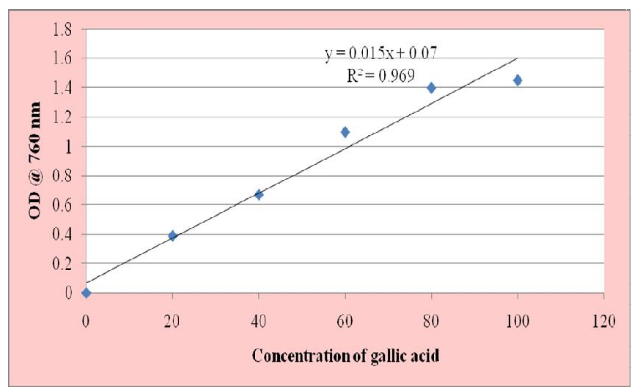

Fig.1. Standard Gallic Acid Curve

Total phenol compounds, as determined by Folin-Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve ($y=0.015x + 0.007$, $R^2 = 0.969$). The Total Polyphenol Content (TPC) of reduced salt spicy sauerkraut was found to be high containing 36.7 mg GAE/100 ml.

Total phenol content was also estimated only in 14 cultivars and the values ranged from 12.58 to 34.41 mg/100 g fresh weight. Amongst the three different cultivated forms of cabbage, red cabbage had higher Vitamin C (24.38 mg/100 g), dl-α-tocopherol (0.261 mg/100 g) and phenolic content (101.30 mg/100 g) as compared to the white cabbage and savoy cabbage (Singh, 2006). Phenolic compounds have multiple additional roles in plants, including attracting insects for seed dispersion and pollination. They are also part of the natural defense system against insects, fungi, viruses and bacteria and they can act as plant hormone controllers. Moreover, in recent years, phenolic compounds have been intensively investigated because of their potential health-promoting effects. They have been reported to possess many useful properties for human health, including anti-inflammatory, enzyme inhibition, antimicrobial, antiallergic, vascular and cytotoxic antitumor activity, but the most important action of phenolics is their antioxidant activity. Furthermore, phenolic compounds possess other properties such as hydrogen peroxide production in the presence of certain metals, the ability to scavenge electrophiles and inhibit nitrosation reactions and chelate metals and, therefore, they act by blocking the initiation of several human diseases. The antioxidant activity of phenolic compounds is related with its chemical structure that confers them redox properties. They can play an important role in adsorbing and neutralizing reactive oxygen species (ROS), quenching singlet and triplet oxygen, or decomposing peroxides. Reactive oxygen species, derived from oxidation processes, are an important part of the defense mechanisms against infection, but excessive generation of free oxygen radicals may damage the tissue. When there is an imbalance between ROS and antioxidant defense mechanisms, the ROS lead to the oxidative modification in cellular membranes or intracellular molecules and result in the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. This oxidative stress has been linked to cancer, aging, atherosclerosis, inflammation and neurodegenerative diseases such as Parkinson's (PA) and Alzheimer's disease (AD). Therefore, antioxidants, such as phenolic compounds, are considered as possible protective agents, reducing the oxidative damage from ROS in the human body and retarding the progress of many chronic diseases as well as the oxidation of low-density lipoproteins (LDL), which is thought to play an important role in atherosclerosis.

Conclusion

Consumers who prefer to consume a healthy product would opt for a low sodium content food with additional health benefits. The use of garlic, pepper during fermentation of cabbage, in a salt reduced environment has effectively increased the production of lactic acid and decreased the pH. Remarkably, the Vitamin K levels of the product were found to be excellent. Additionally, spicy sauerkraut is rich in carbohydrate, flavanoids, quinines, terpenoids, and phenol. Therefore, consumption of spicy sauerkraut could be a tastier and healthier food choice to achieve optimal nutrition goals among consumers.

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