CHANGES IN THE CONCENTRATION OF CARBOHYDRATES, ORGANIC ACIDS AND AMINO ACIDS OF SOAKED PEANUTS WITH LACTIC ACID BACTERIA

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ABSTRACTS. *Peanuts were soaked for 24 hours in water at 30℃ in preparation for sauce production. Soaking was conducted under conditions that cause normal microbial fermentation and in the presence of Pediococcus halophilus. The aim of this study is to investigate the changes in carbohydrate, organic acid and amino acid concentrations during short period soaking under normal fermentation and with inoculated lactic acid bacteria, Pediococcus halophilus. Changes in the concentration of carbohydrates, organic acids and amino acids of the soaked peanuts were determined. The principal sugar present in the peanut was sucrose, 46.33 mg g^{-1} peanut (DM). Sucrose concentration dropped during soaking although at a slower rate in the inoculated peanut. Acetic, lactic, citric and oxalic acids were detected. Acetic acid was the main organic acid in the uninoculated peanut whereas acetic and lactic acids were prominent in the inoculated peanut. Higher concentrations of aspartic acid, glutamic acid, valine, isoleucine and phenylalanine were found in the inoculated beans after 24 hours soaking although soaking for18 hours was found to yield an optimum amino acid concentration. The presence of organic acids and principle amino acids produced during controlled fermentation contributed to the flavour enhancing effect.*

KEYWORDS. Peanut sauce, lactic acid bacteria, amino acid production, organic acid production

INTRODUCTION

Peanut is a popular ingredient used in the preparation of sauces and gravies. In some preparations, the nuts are preliminarily soaked overnight and thus undergo a mild fermentation. Through this process the beany flavour is removed and an enhanced savoury characteristic is achieved (Wang *et al*., 1979; Song *et al.*, 2009). Soaking the legumes in water is the essential preliminary process for their preparation (Mulyowidarso *et al.*, 2008). Besides reducing cooking time, soaking improves the organoleptic properties, nutritional value and texture of the product (Wang *et al*., 1979; Omafuvbe *et al.*, 2007). However Kailasapathy *et al*., (1985) observed that the soak water of wing beans contained reducedsugars and amino acid, indicating that some of the nutritional matter diffused out during soaking. Soaking influences the content of potential flavour precursors whereby nitrogenous compound including water soluble amino acids and short-chain fatty acid diffused out from the tissues during soaking (Drumm *et al*., 1990). Soaking also contributes to reduction of monosaccharides and oligosaccharides (Silva & Braga, 1982; Drumm *et al*., 1990). The soaking process is normally accompanied by hydration (Mulyowidarso *et al.*, 2008) and softening of the beans which facilitates leaching out of beans' components (Lo *et al.*, 1968) and activation of endogenous enzyme (Wang *et al*., 1979). Such components would encourage microbial growth and possibly encourage certain species to grow selectively.

The soaking process of various legumes has been described, such as winged beans (*Psophocarpus tetragonolobus*) (Kailasapathy *et al*., 1985; Buckle & Samudi, 1990), *Phaseolus vulgaris* (Drumm *et al*., 1990; Quast & Silva, 1977), Mung bean (Sattar *et al*., 1988), navy bean (Kakade & Evans, 1966) and soya bean (Wang *et al*., 1979; Mulyowidarso *et al*., 2008). The aspects studied involved solid losses, quality and cooking rate, rate of rehydration, nutritional value and antinutrients present.

Addition of cultures to aid the desired fermentation is beneficial and is practised widely as in tempe (Mulyowidarso *et al*. 2008), oncom (Beuchat, 1976) and soy sauce (Steinkraus, 1983). The main contribution of these cultures is as flavour enhancers mainly contributed by the organic acids and principle amino acids produced during controlled fermentation.

Although soaked peanut is widely used as a food ingredient and its chemical composition is known, changes in carbohydrate, organic acids and amino acid composition profiles during the short soaking duration have not been elucidated.

The aim of this study is to investigate the changes in carbohydrates, organic acids and amino acids concentrations during short period soaking under normal fermentation and with the addition of inoculated lactic acid bacteria, *Pediococcus halophilus*.

MATERIALS AND METHODS

Soaking of Peanuts

The peanuts, *Arachis hypogaea* (200 g) were mixed with 667 mL dechlorinated tap water in a sterile 1 litre flask. *Pediococcus halophilus* was inoculated in the soak-water to give an initial population of 10^4 mL^{-1}. Control experiments were conducted where the beans were soaked according to the standard procedure except that the inoculum was not used. The flasks were then plugged with sterile cotton wool, covered with aluminium foil and placed in an orbital shaker at the running speed of 50 rpm. Sampling was done at 0, 6, 12, 18 and 24 h. Each type of sampling experiment and analysis was conducted in triplicate.

Determination of Carbohydrates

Carbohydrate extraction was done according to the method of Mulyowidarso *et al*. (2008) with slight modifications. Soaked beans (60g) were removed and homogenised with 5 mL distilled water for 3 minutes in a Waring blender. The homogenate (5 g) was mixed with 100 mL 80% ethanol in a 250 mL flask and boiled under reflux for 25 min at 80 °C. Ethanol extract was then filtered and the peanut residue was further washed with 50mL 80% ethanol twice. The extract was pooled and placed in a centrifuge tube. Several drops of 10% lead acetate were added to precipitate the protein. The extract was then centrifuged at 3500 x g for 30 min. While the supernatant was collected, the residue was further washed with 15 mL 80% ethanol twice. The pooled ethanolic extract was evaporated in a rotary evaporator at 60 °C for 30 min. The concentrated extract was then reconstituted with 50 mL distilled water, treated with 3 g Kieselguhr and then centrifuged at 3500 X g for 30 min.. The supernatant was filtered through Sep Pak Catridge twice followed by Millipore filter (0.45μm) before being injected into an HPLC column.

Sugar concentration was determined by HPLC method using Water^{TM} Liquid Chromatography System involving Sep Pak Catridge, refractive index detector and Sugar-Pak column 300 X 6.5 mm(i.d.), packed with micro particulate cation exchanger resin. The column was eluted at 0.5 mL min^{-1} and deionised water was used as a mobile phase. Individual carbohydrate concentration was determined by referring to the eluted standard solution for glucose, fructose and sucrose.

Determination of Organic Acids

Soaked peanuts (10 g) were defatted using chloroform-methanol mixture (2:1 v/v) as described by Mulyowidarso *et al*. (2008). The sample was then homogenised using Jauke and Kunkel Ultra-Turrax T25 homogeniser at 8000 rpm for 5 min. and filtered quantitatively using filter paper (Whatman No. 5). Peanut residue was mixed with 30 mL distilled water and sonicated for 3 min. The mixture was then centrifuged at 6000 x g for 15 min at 5ºC and the sediment was washed twice with 10 mL distilled water. The supernatant was pooled and treated with 0.1% trichloroacetic acid before being passed through Sep-Pak C-18 column and Millipore filter (0.45μm) prior to HPLC analysis.

Individual organic acid concentration was determined using HPLC consisting of UV detector operated at 210 nm. Organic acids were eluted at ambient temperature using 0.10 mol L^{-1} sulphuric acid as mobile phase at a flow rate of 0.5 mL min^{-1}. Individual organic acid concentration was determined with reference to the elution of standard organic acids (acetic, lactic, citric and oxalic acids).

Determination of Amino Acids

Amino acids were determined using HPLC employing a PICO-TAG amino acid analysis system. The relevant system consisted of two pumps A and B (Waters model 510), injector (Waters model 710B), absorption detector (Waters model 440), and PICO.TAG column (Waters). This method involved sample hydrolysis and pre-column derivatisation. Ground peanut weighing 0.5 g was mixed into a screw-cap test-tube containing 15 mL of 0.6 molL^{-1} HCL. The test-tube was transferred into a canister which was then heated in an oven at 110°C for 24 hours. Hydrolysed sample was filtered using Whatman filter paper (No. 4). The filtrate was made-up to 50 mL with deionised water and was further filtered with Millex filter. An aliqout (10 μL) was transferred into a small tube and dried in a PICO-TAG dryer at 200 milliTor vacuum. Drying solution (40μL), consisting of 2:2:1 mixture of methanol, HPLC grade water and Triethyl amine (TEA) was added to the dried sample and the mixture was shaken for 10 minutes. The tube was then vacuumed again at 200 milliTor. Derivatisation reagent (20 μL), consisting of a mixture of 400 μL methanol: water (7:1), 50 μL TEA and 50 μL Phenylisothiocynate was added to the tube and shaken for 10 minutes prior to drying as described earlier. Sample diluent (100 μL) was added to the tube and mixed thoroughly. A volume of 20 μL of the prepared sample was injected into the HPLC. The amino acid profile was determined by referring to the eluted individual amino acid standards i.e. L-alanine, L-arginine, L-aspatic acid, L-cysteine, L-glutamic acid, glycine, L-histidine, L-isoluecine, L-leusine, L-lycine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine (Sigma).

RESULTS AND DISCUSSION

Changes in Carbohydrate Content

Carbohydrate extracted using 80% ethanol represents mono, di and oligosaccharides (Mulyowidarso *et al*., 2008). In this study, the extracted carbohydrates were sucrose, glucose and fructose. The peanut was found to contain 46.33 mg sucrose, 0.17 mg glucose and 0.22 mg fructose per gram peanut (dry weight). This is comparable to the values quoted by Adsule *et al*., (1989) who found that the sucrose, glucose and fructose contents were in the range of 27.8 - 37.5, 0.19 - 0.23 and 0.09 - 0.11 mg per g peanut (dry weight), respectively, in different *Arachis hypogaea* cultivars.

The concentration of the main sugar in peanut, sucrose, was found to decrease progressively during soaking for both types of fermentations studied. A slower decline

(Figure 1) was noted for inoculated peanuts than control peanuts initially but the concentration stabilised after 12 h incubation period.

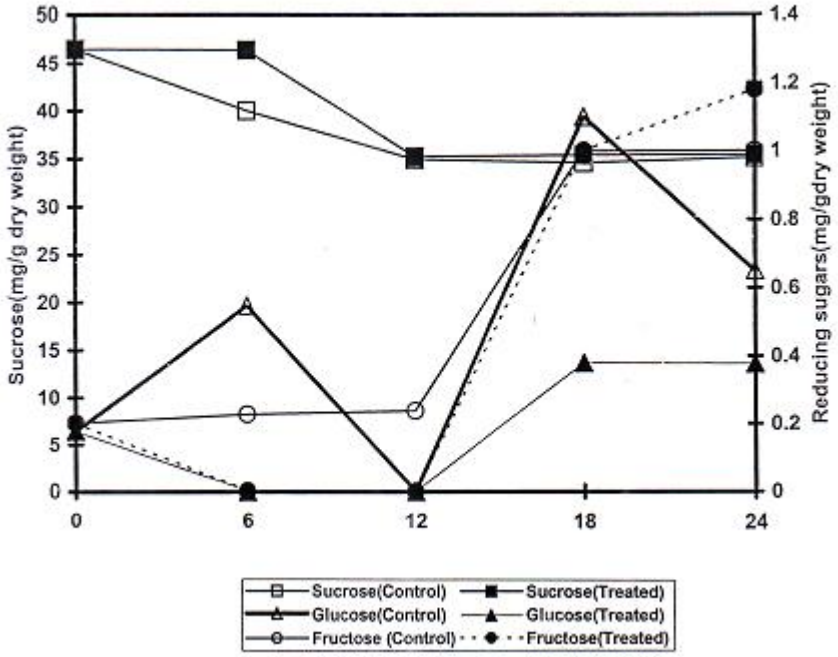

Figure 1. Sugar concentration of peanut during soaking.

Many factors are known to cause the drop in sucrose concentration during soaking of peanut, namely leaching (Silva & Braga, 1982; Drumm *et al*., 1990), endogenous hydrolytic enzymes activities and microbial fermentation. Hydration of peanut initiates germination, where the endogenous sucrose degrading enzyme, invertase degrades sucrose to glucose and fructose (Wang *et al*., 1979, Kuo *et al*., 1988). Invertase is also produced by microbial species present in the soak water and aids the hydrolysis of sucrose (Fogarty, 1983).

Glucose concentration was found to increase after 6 h fermentation (0.58 mg g^{-1} dry weight) for the untreated peanuts but dropped thereafter and was not detected at 12 h. However it peaked again at 18 h, with a concentration of 1.10 mg glucose g^{-1} dry weight peanuts. In contrast, an abrupt decline in glucose concentration occurred for the inoculated peanut, was undetected at 6 and 12 h, to be detected again only after 12 h, with a maximum concentration of 0.37 mg g^{-1} dry weight after 18 h soaking. Similar trends were observed with fructose concentration where an abrupt drop to undetectable concentration was noted with the inoculated peanut at 6 and 12 h soaking, but increasing rapidly thereafter to a value of 1.20 mg g^{-1} dry weight.

Microorganisms ferment sugars and produce acid, reducing pH to 5.0 at 18 h incubation period, thus influencing the invertase activity in the peanut (Mulyowidarso *et al*., 2008). The optimum pH of invertase is between 4.5 - 5.5 (Whitaker, 1972). The higher glucose and fructose content of the soaked peanuts compared to the original peanut samples suggests that hydrolysis of carbohydrate, particularly sucrose to glucose and fructose was higher during soaking. Fructose, especially, was elevated up to fivefold for both the natural and inoculated fermentations at 18 h. This is of significance to the flavour as it also has a higher relative sweetness compared to sucrose and glucose.

Changes in Organic Acids Content

In the natural fermentation, acetic acid concentration increased at 6 h soaking period but decreased gradually throughout 24 h soaking (Figure 2). Lactic acid was found to decrease throughout the fermentation course. However fluctuation in the citric acid and oxalic acid content was observed upon soaking. In lactic acid fermentation, acetic acid concentration decreased at 6 h, increased slightly at 12 h and remained fairly constant until 24 h soaking. Lactic acid concentration showed a two fold increase at 6 h followed by a slight drop at 12 h and continued to drop thereafter. However oxalic acid concentration did not show much change throughout the soaking period.

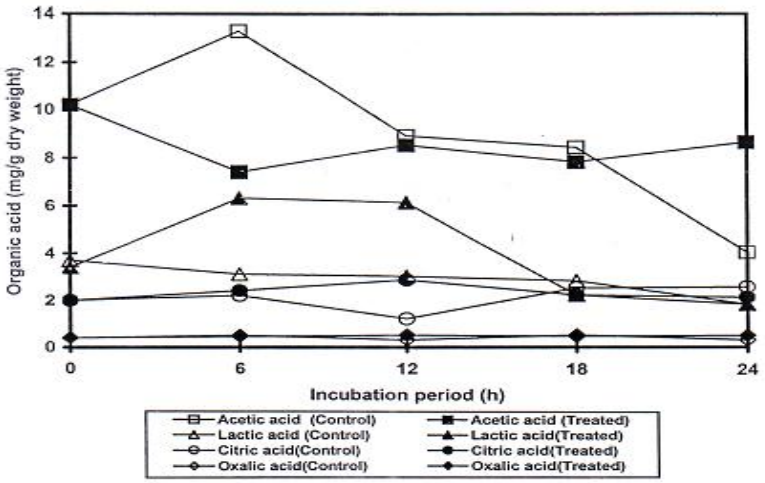

Figure 2. Organic acids concentration of peanuts during soaking.

Changes in Amino Acids Content

Changes in the concentration of amino acids of the uninoculated and the inoculated beans during soaking are shown in Table 1. After 24h soaking, the concentration of aspartic acid, glutamic acid, alanine, valine, methionine, cysteine, isoleucine and phenylalanine were found to be higher in the treated samples. It was also noted that aspartic acid, glutamic acid, serine, glycine, arginine, threonine and lycine yielded the highest concentration at the 18 h soaking period with the inoculated beans, coinciding with peak microbial counts in the sample. Apart from the endogenous enzyme activities, microorganisms in the peanut and soak water also produce protease (Kailasapathy *et al*., 1985; Rasch *et al*., 2005).

Table 1. Amino acid concentration of peanuts during soaking.

Amino acid (mg/g dry weight)	Soaking period (h)									
	0		6		12		18		24	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Aspartic acid	21.32	21.32	21.84	11.86	14.42	8.47	12.35	20.82	11.36	17.99
Glutamic acid	50.29	50.29	30.50	23.92	20.70	17.56	25.54	33.87	25.72	32.06
Serine	12.86	12.86	9.11	6.40	10.04	7.62	6.31	11.07	8.07	3.20
Glycine	12.33	12.33	7.80	5.35	10.19	7.57	8.19	10.22	8.50	4.32
Histidine	5.54	5.54	2.73	1.43	2.60	1.47	3.53	3.18	2.60	tr
Arginine	33.75	33.75	20.35	16.40	15.97	13.92	20.86	22.22	20.03	15.17
Threonine	6.69	6.69	3.86	3.11	3.52	3.08	3.77	21.37	3.63	2.85
Alanine	8.30	8.30	5.67	4.52	11.2	9.67	4.72	6.24	5.43	6.49
Proline	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
Tyrosine	11.38	11.38	6.17	4.17	8.66	6.33	6.92	9.25	13.40	5.82
Valine	8.55	8.55	9.38	2.62	13.85	4.19	4.20	5.90	4.22	5.17
Methionine	1.93	1.93	0.95	0.99	0.45	0.51	1.24	1.11	0.90	1.23
Cysteine	2.10	2.10	1.03	1.09	0.49	0.56	1.35	1.21	0.98	1.34
Isoleucine	14.07	14.07	4.35	3.55	2.96	2.61	3.66	7.21	4.00	8.38
Leucine	15.60	15.60	7.98	7.48	4.20	4.27	12.38	11.52	5.78	7.16
Phenylalanine	12.21	12.21	4.69	3.70	4.15	3.54	5.04	6.22	3.90	4.72
Lysine	25.75	25.75	30.98	28.33	32.55	32.22	41.32	41.84	40.72	39.81

Note: tr; trace

For both fermentations, amino acid content was found to be lower in the soaked peanut. The drop could possibly be due to the leaching of free amino acids which were very soluble in the soak water. Hydration of peanuts during soaking also initiates germination and activates endogenous proteolytic enzymes in the beans to breakdown protein to lower molecular weight peptides and amino acids. Glutamic acid, an important flavour precursor, was found to be higher in inoculated beans at 18 and 24 h. The peanuts under lactic acid fermentation yield higher acetic acid and reducing sugars, thus providing more substrate for metabolism of *P. halophilus*. Peanuts also contain biotin, which is required for glutamic acid production (Higgin *et al*., 1985).

CONCLUSION

The time of soaking influences the composition of the peanuts. Controlled soaking of peanuts for 18 h with the presence of *P. halophilus* was found to yield a higher total amino acid content. Glutamic acid concentration, a flavour potentiator was also found to be higher in the cultured peanuts. Prolonged incubation of the cultured peanuts to 24 h yielded higher acetic acid and fructose, which are significant organoleptic contributors in peanut sauce formulation.

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