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EFFECT OF FERMENTATION ON PROTEIN DIGESTIBILITY OF SOYBEAN AND SWEET POTATO BLENDS: *ASPERGILLUS ORYZAE VS. LACTOBACILLUS PLANTARUM*

KIPLAMAI F. K. [1], SHALO P.L. [2], MAHUNGU S.M. [2] and TUITOEK P.J. [3],

[1] *Department of Exercise, Recreation and Sport Science, Kenyatta University,* [2]*Department of Dairy and Food Science and Technology, Egerton University,* [3] *Department of Foods Nutrition and Dietetics, Egerton University,*

ABSTRACT

The improvement of nutritional quality of foods through fermentation has been practiced for long. Fermentation imparts desirable characteristics to products and makes them more utilizable nutrient sources than the unfermented products. Sweet potatoes have been under utilized industrially and are mainly used at household level. Soybeans are rich in proteins but their full utilization has been hampered by their anti-nutritional properties which are destroyed by heating and fermentation. The purpose of this study was to produce suitable blends of soybean and sweet potato flours targeted at alleviating protein related malnutrition. The quality characteristics of the fermented and unfermented soybean and sweet potato composite flours were compared. The fermentation with Lactobacillus plantarum was at 37oC for 168 hours with sampling every 24 hours. The Aspergillus oryzae fermentation was performed at 25oC for six weeks with samples taken weekly for laboratory analysis. In-vitro protein digestibility was determined enzymatically. The data obtained was subjected to General Linear Model (GLM) of the Statistical Analysis System (SAS). Protein digestibility was improved by an average 4% (p<0.05) in the bacterial fermented composites and by an average of 5.5% (p<0.05) in the composite flours fermented with the mould. The composite containing 50% soybean and 50% sweet potato reflected higher protein digestibility than the composite with 25% soybean and 75% sweet potato. Fermentation significantly increased protein digestibility but an initial drop was observed in the higher (50% soybean and 50% sweet potato) protein composite for both bacterial and mould fermentations. Optimum protein digestibility improvement was achieved thus a baseline for development of suitable blends targeting protein malnutrition.

INTRODUCTION

The aim of food biotechnology is to exploit natural food resources as efficiently and profitably as possible. Adequate and economical processing, prolongation of shelf life, improvement of safety and nutritive value, appropriate packaging, and maximum consumer appeal are key prerequisites to achieve this aim (Gaden *et al.*, 1992). Fermentation is the oldest form of food biotechnology. The traditional fermentation processes serve several functions including the enrichment of food substrates with amino acids, fatty acids, vitamins and poly amines. Fermentation with *Lactobacilli*, for example, increases the availability, digestibility and assimilation of various nutrients to the body (Vijaya *et al.*, 2002). Fermentation is the process in which micro-organisms multiply in the culture medium, consume mostly carbohydrate component, transform the composition of the culture medium and in the process enrich it with products of their metabolism. Vitamins, amino acids and fatty acids are formed, thus improve the nutritive value of foods. During fermentation, the protein structure undergoes desired basic structural changes (Rechcigl, 1986). In many African countries, protein-energy malnutrition is a big problem and fermented vegetable proteins have a great potential as protein sources and basic ingredient in food supplements.

Nutritionally modified food rations have been used in various hospices, malnutrition rehabilitation centers, refugee camps and in community-based organizations in alleviating malnutrition. Malnutrition is often associated with increased incidences of diseases which reduce nutrient intake while increasing the need for macro- and micro-nutrients. Modification of food biopolymers such as carbohydrates and proteins is often achieved through various food processing steps such as heating and fermentation. During fermentation the action of enzymes from the mould and bacterial culture, such as, *Aspergillus oryzae* and *Lactobacillus plantarum* respectively yield products that are microbiologically safe with improved palatability and digestibility. Other important attributes associated with fermentation include improved keeping quality, nutritional value, ease of preparation and increased yield of the edible fraction of the food material.

Address for correspondence: F. K. Kiplamai
Department of Exercise, Recreation and Sport Science,
Kenyatta University
P. O Box 43844, Nairobi. Kenya
Email: f.kiplamai@yahoo.com

In most tropical countries, foods are obtained mainly from vegetable sources (cereals, legumes, roots and tubers). These foods are usually presented as thick porridges for adults and gruels for young children. These are made using a large amount of water thereby resulting in low nutrient density foods. The protein quality in such products is usually lower than the animal protein due to the presence of fibres, phytates and tannins (Sanni *et al.*, 1999). It is therefore important to improve the potential of a food in relation to its nutrient density through fermentation thus alleviate the factors of malnutrition inherent in the food.

In a study by Korhonen *et al.* (1998), plant proteins especially those from soybeans were found to contain potential bioactive peptide chains which are liberated during fermentation. The enzymes evolved during fermentation include the extra cellular and intracellular enzymes. Digestibility is affected by the polymer structure. Through fermentation the polymer structure is opened up and made more amenable to the digestive enzymes and subsequently quick absorption. The tediousness of chewing is reduced leading to overall increase in palatability.

Chinnock (2001) showed that the foods consumed by malnourished individuals in Africa are low in energy and nutrient density. Therefore, fermentation improves the availability of nutrients thereby contributing to the alleviation of malnutrition. In this study, the fermentation process was studied in relation to the nutritional improvement of soybean and sweet potato composite flours. The focus was mainly the action of *L. plantarum* and *A. oryzae* fermentation on the digestibility of the protein fraction of the composite flours and the hygiene and safety aspects of fermented flours.

The nutrient and energy intake by malnourished individuals is much lower than the body requirements. This inadequacy in intake of food is further aggravated by nutrient mal-absorption due to body metabolic alterations, which are conditions faced by poor people in developing countries. Increasing the intake of highly palatable, easily digested, absorbed and high nutrient formulations counteracts this caloric and protein deficiencies. Soybeans are known to have anti-nutrients which are reduced during fermentation thus increased bioavailability of these nutrients. This is especially so in areas where arable farming is the primary occupation and in rehabilitation centres and refugee camps where malnutrition is rampant.

The general objective of this study was to determine the effects of fermentation, using different organisms, on the nutrient enrichment of composites formulated using different ratios of soybeans and sweet potato flours. The specific objective was to determine the pattern of changes in protein digestibility caused by fermentation.

MATERIALS AND METHODS

Sweet potato tubers (Variety SPK 004) were procured from the Kenya Agricultural Research Institute in Kakamega (Kenya). Soybeans (Glycine Wightii sub- spp petitiana) were purchased from the local market in Nakuru town (Kenya) in a single lot. The experiments were carried out in the Dairy, Food science and Technology Department and in the Department of Animal Science at Egerton University. The fermentations were set up in a Completely Randomized Design (CRD). The bacterial fermentations had two treatments (Comp A and Comp B) and five periods (24, 48, 72, 96 and 168 hours) while the mould fermentations had two treatments (Comp A and Comp B) and six periods (1, 2, 3, 4, 5 and 6 weeks).

Sample preparation

The soybeans were sorted by hand to obtain non-defective seeds and then soaked in water for 12 hours in water at ambient temperature (23-25°C). They were then de-hulled and cooked by boiling in water until the soybean could be crushed between the middle finger and thumb. They were sun dried at about 50–60° C to approximately 12% moisture content and milled using a hammer mill (Christy Hunt) to obtain soy flour.

The sweet potatoes were scrubbed to remove soil and the skin without removing the outer layer of the tuber flesh. They were chopped into small cubes (2 cm by 2 cm) and cooked in boiling water (94°C) for 27 minutes as described by Paul and Southgate (1978). They were sun dried to approximately 12% moisture content and milled using a hammer mill to obtain sweet potato flour.

Microbial cultures

Spores of *Aspergillus oryzae* and lyophilized cultures of *Lactobacillus plantarum* (NTCC 1752) were obtained from G.E.M Cultures (30301 Sherwood road, Fort Bragg, CA 95437 USA). The lyophilized cells of *L. plantarum* were transferred to sterilized nutrient broth medium under aseptic conditions and incubated at 37°C for 24 hours, tested for activity and then stored in a refrigerator at 4°C. The activity of the *L. plantarum* culture was tested using lactic acid production as described by Sandine (1979). The following procedure was adopted: Skim milk powder was reconstituted (10% solids) and sterilized by heating at 94°C for 5 minutes. It was then inoculated with 3% of the *L. plantarum* culture (v/v) and incubated at 37°C for 2.5 hours and 3.5hours. Titration was done using 0.1N NaOH and the activity calculated using formula 1. The difference in the two lactic acid values calculated as a percentage was used as a measure of the level of starter activity.

% Lactic acid = ml NaOH x 0.1...................... Formula 1

The *Aspergillus oryzae* spores were mixed with soybean flour, which had been subjected to dry-heat on a steel pan to sterilize it as per the activation procedures provided by G.E.M cultures. This mixture (spores: soybean flour, 1:2; as per supplier) was used to inoculate the composite flours as the activated spores. Growth of the mould culture (cotton-like whitish appearance) after 48 hours at room temperature was indicative of an active culture.

DEVELOPMENT OF COMPOSITE FLOURS

Soybean flour and Sweet potato flour were mixed in the ratio of 1:1 (composite A) and 1:3 (composite B), respectively on dry weight basis. The rationale of the choice of these composite ratios was to allow for comparison of the effects caused by reducing the ratio of the protein source (soybean) would have on the attributes studied during the fermentation. Each of the composite flours (100gm) was mixed with distilled water (500ml) and stirred sufficiently to form homogeneous slurries. These slurries were then inoculated with 2% (v/v) liquid cultures of *Lactobacillus plantarum* (24 hour preserved culture). These slurries were incubated at 37° C for 7 days during which period samples were collected after every 24 hours for analysis. This was to establish the changes in the composites after each day of fermentation as done by Yousif and Abdullahi (2000). The samples were oven (Electrolux) dried at 60°C to 12% moisture content and packaged in transparent polyethylene bags to await analysis.

Other batches of the composite flours (100 gm) were each mixed sufficiently with distilled water (300 ml) to form thick pastes, which were inoculated with 8% (w/w) of the activated spores of *Aspergillus oryzae*. Parallel furrows 2 inches apart and 1 inch deep, were made across the entire surface of the paste to make the medium aerobic and reduce the possibility of development of hot spots. They were incubated at room temperature (25° C) for 48 hours after which the pastes had a faintly yeasty smell and whitish cotton-like surface appearance due to mould growth. The mouldy pastes were then mixed with 18% salt to stop their growth. Since optimum enzyme production had been achieved and sporulation had not occurred. The pastes were further incubated at room temperature for six weeks (maturation period) during which period sampling was done weekly. These samples were oven dried to a moisture content of 12 % and packaged in polythene bags for analysis. The sampling for the mould fermentations was done weekly because it is only the exogenous enzymes that are produced and utilized in the mould fermentation unlike the bacterial fermentation where sampling was done daily because both endogenous and exogenous enzymes are produced in the fermentation process.

The fermentations were done in duplicate and the samples from the first fermentation analyzed in triplicate while the samples from the second fermentation were done in duplicate due to laboratory limitations. This gave five independent determinations while the unfermented composites (cooked and uncooked soybean) flours served as controls.

In-vitro Assay for protein digestibility

The *in-vitro* Protein digestibility for all the samples was carried out as described by FAO/WHO (1991): Sufficient amounts of porcine pancreatic trypsin (type IX, sigma 7-014), bovine pancreatic ▯-chymotrypsin (type II, sigma C-4129), and porcine intestinal peptidase (grade K, sigma

P-7520) were dissolved in distilled water to give 23,100; 186 and 0.052 units per ml, respectively. The enzyme suspension was adjusted to pH 8.0 and maintained for two minutes at 37° C, then transferred to an ice bath and kept at 0° C to await use. Sodium caseinate suspension (10 ml) was placed in the reaction vessel, warmed to 37°C and pH adjusted to 8.0 and maintained for 5 - 10 minutes before 1 ml of the 3-enzyme solution was added. While stirring, the amount of 0.1N NaOH required to maintain the pH at 7.98 for 10 minutes was added (FAO/WHO 1991).

The *in-vitro* Protein Digestibility (IVPD) of the sodium caseinate control was calculated using formula 2. The digestibility of the test samples was done as described using sample amounts containing 10 mg N dissolved in 10 ml distilled water. Sodium caseinate was used as a control to give the laboratory correction factor as shown in Formula 3. This laboratory correction factor was used to adjust the digestibilities of the test samples.

IVPD = 76.14 + 47.77B ....Formula 2
(B – ml of 0.1N NaOH used)

Laboratory correction factor = 100 /Sodium caseinate digestibility...........Formula 3

STATISTICAL ANALYSIS

The determinations were done in duplicate and the figures averaged. The data was analyzed through the Analysis of Variance (ANOVA), using General Linear Model procedure of the Statistical Analysis System (SAS) with Duncan's Multiple Range Test used to compare means because the means being compared are more than two (SAS institute 2000). A P-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The activity (acidity calculated as % lactic acid) of the *L. plantarum* (24 hours old culture) was found to be 0.14% ± 0.01 lactic acid at 2.5 hours and 0.24% ± 0.01 lactic acid after 3.5 hours, which indicates that the starter culture was sufficiently active. Sandine (1979) showed that a difference of 0.08% to 1% lactic acid between these two readings is indicative of an active culture. The difference in the lactic acid in this case was 0.1 %.

The means of IVPD of the composites fermented using *Lactobacillus plantarum* are presented in Table 1 and that fermented using *A. oryzae* in Table 2.

The means of IVPD showed that there were significant differences (at p<0.05) due to the effect of fermentation with *L. plantarum* and *A. oryzae*. There was a significant difference between the IVPD of the two composites with an overall average of 94.4% and 92.0% for composite A and B, respectively. Composite A fermented with *L. plantarum* for 168 hours ranked highest in IVPD when ranked using the Duncan's Multiple Range Test (DMRT) and it was significantly different from all the others.

Soaking; dehulling and cooking resulted in a significant increase in the IVPD of soybean from 83.62% for raw soybean flour to 89.17% for the cooked soybean flour. This increase suggests that the processing methods cause structural changes in the soybean proteins content (β-conglycinin and glycinin). Sindhu and Khetarpaul (2001) showed that autoclaving causes partial breakdown of protein, which results in an increase in the *in-vitro* protein digestibility.

Table 1. Nitrogen, %IVPD and pH of samples fermented using *L. plantarum.*

Sample	% IVPD	
Time (hrs)	Comp A	Comp B
0	93.3 ± 0.2^a	87.8 ± 0.6^a
24	93.0 ± 0.4^a	88.3 ± 0.3^b
48	93.8 ± 0.4^b	88.8 ± 0.3^b
72	94.9 ± 0.4^c	89.7 ± 0.2^c
96	95.7 ± 0.5^d	90.5 ± 0.3^d
168	97.2 ± 0.3^e	91.2 ± 0.4^e

Means in the same column with the same letter are not significantly different (P>0.05).
Comp A – (Composite A) Soybeans: Sweet potatoes in the ratio 1:1
Comp B – (Composite B) Soybeans: Sweet potatoes in the ratio 1:3
Note: The choice of the composite flours was to provide a comparison of effects due to fermentation when the amount of the protein source is changed.

Table 2 Nitrogen, %IVPD and pH of samples fermented using A. oryzae.

Time (Weeks)	% IVPD	
	Comp A	Comp B
0	93.6 ± 0.2^{ab}	87.8 ± 0.6^a
1	92.4 ± 0.3^a	92.2 ± 0.3^{ab}
2	92.8 ± 0.5^{ab}	92.8 ± 0.4^{bc}
3	93.6 ± 0.5^b	93.3 ± 0.7^{cd}
4	94.4 ± 0.7^c	94.1 ± 0.4^{de}
5	94.9 ± 0.5^{cd}	94.9 ± 0.7^e
6	95.6 ± 0.7^d	96.1 ± 0.4^f

Means in the same column with the same letter are not significantly different (P>0.05).

The IVPD of the unfermented composites was 93.3 ± 0.2 and 87.8 ± 0.6 for composite A and B respectively. The difference in the unfermented composites is attributed to the variation of the ratio of the composite ingredients, since composite A has a higher amount of soybean flour compared to composite B. However, the differences in the IVPD of the soybean flour and the unfermented composites could be attributed to the addition of sweet potatoes whose protein content is very low (0.19mgN/g) compared to that of soybeans (75.77mg N/g).
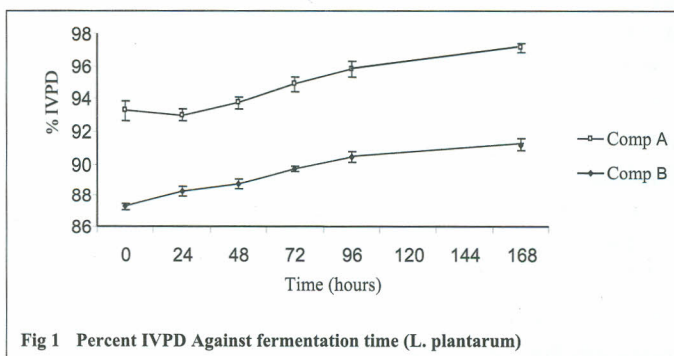

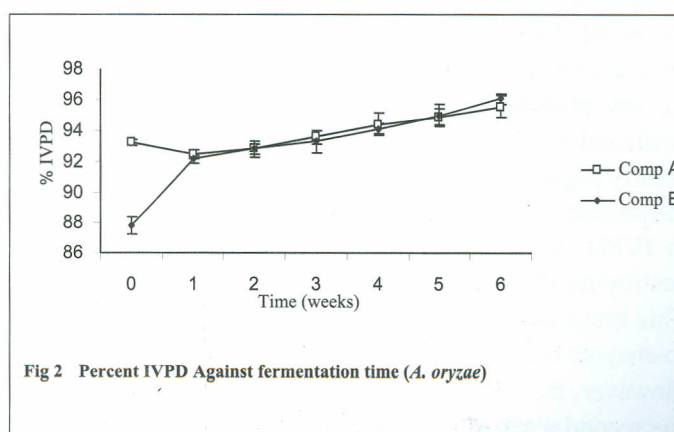Fig 1 Percent IVPD Against fermentation time (L. plantarum)

Fig 2 Percent IVPD Against fermentation time (*A. oryzae*)

There was a slight insignificant decrease in IVPD of composite A in both the first 24 hours of *L. plantarum* fermentation and first one week of *A. oryzae* fermentation as shown in Fig 1 and Fig 2 respectively. This may be attributed to the presence of high amounts of proteases produced by the microorganisms due to the high protein content of the composite.

Saleh *et al.,* (2003) showed that carbohydrases break down Non Starch Polysaccharides (NSP) leading to an increase in IVPD. They further showed that proteases digest carbohydrases, thus justifying this decrease in IVPD. It was also observed that composite B has a lower average IVPD than composite A in the bacterial fermentation (Table 1). This can be attributed to the masking effects caused by the presence of high amount of sweet potato flour. This result concurs with that by Satterlee *et al.,* (1981) and indicates that the presence of non-protein components cause masking or complex formation thus lowering digestibility of protein.

Upon bacterial fermentation of the composites there was a steady significant increase in the IVPD with time through out the fermentation. This was found to correspond with the study by Sindhu and Khetarpaul (2001) which showed that fermentation of an indigenous food mixture using *L. plantarum* and *S. boulardii* improved the protein

digestibility significantly. Giami (2004) also showed that the IVPD of fermented fluted pumpkin seeds increased in the first 108 hours of bacterial fermentation but no significant change was noted thereafter. The structural changes undergone by the insoluble protein during fermentation made it more susceptible to enzymatic attack. This partly explains the increase in IVPD observed in this study. Findings in this study also concur with a study by Granito et al (2002), in which it was found that despite a decrease in protein nitrogen content of the naturally fermented beans (Phaseolus vulgaricus), the IVPD increased irrespective of the fermentation conditions. Moneim et al (1995) showed that the IVPD of protein fractions of low tannin sorghum cultivar increased due to the effect of bacterial fermentation.

The mould fermented samples (Fig. 2) exhibited varied trends in the IVPD of the composites initially with composite A decreasing slightly while composite B sharply increased. The reduction in the IVPD of composite A has been shown, to be due to the digestion of the carbohydrases by the proteases. The trend in composite B can be attributed to the break down of the masking compounds mainly sugars present in this composite, thereby exposing the proteins to enzymatic attack thus depicting an increase in IVPD. It is also seen that the mould is superior in destroying these masking components than the bacteria. This break down exposes the protein portion of soybean to enzyme digestion thus resulting in a high IVPD value. However, the trend for both composites was similar from the second week of fermentation with a steady increase of the IVPD until the end of the 6 weeks of fermentation. The mould enzymes are higher in quantity at the beginning of fermentation. Further enzyme production is stopped after optimal enzyme production and before sporulation by addition of 18% salt. Due to enzyme inhibition factors the enzymes continually decrease in quantity and activity as the fermentation progresses. This explains the trends in IVPD after the first week of fermentation in both composites. In Koji (Japanese soy sauce) making, the maturation duration ranges between four and twelve months since it is known that the enzymatic action acts continuously and the desired quality characteristics are attained after the prolonged fermentation (Nakadai et al.,

1972). In this study, the IVPD of the mould fermented composites indicates a linear relation (Fig 2) to the fermentation time, which depicts that the increase could continue even after six weeks of product maturation.

It is worthwhile to note that the fermentation of soybean and sweet potato composite flours improves protein digestibility despite using different starter cultures. The use of L. plantarum as a starter organism is superior to A. oryzae with respect to IVPD, which has been shown to increase steadily in both composites. This has an economic advantage, in that bacterial fermentation for one week achieved an improvement of IVPD of about 3.7% for both composites while mould fermentation achieved an average increase of 5.5% after six weeks of fermentation. In comparison the trends of IVPD for the composites, it was noted that composite A has a better IVPD than composite B in both the bacterial and mould fermentation. This can be attributed to the intrinsic properties and components contributed by the amount of sweet potato flour in the composite, which cause masking that showed low IVPD. The activity of the bacterial fermentation was generally higher in the high protein sample compared to the mould fermentation. Soaking, de-hulling, cooking and fermentation of soybean and soy based products have been shown in this study to be basic processing steps responsible for improvement of IVPD due to their ability to remove or reduce intrinsic and extrinsic factors that depress the IVPD while imparting factors that improve protein digestibility.

CONCLUSION

It can be concluded from the findings of this study that fermentation improves the nutritional quality of the soybean/sweet potato composite flours. The in-vitro protein digestibility was improved irrespective of the organism or composite used, but the bacterial culture exhibited higher overall improvement. The products of these fermentations can therefore be applied in malnutrition alleviation programmes and in development of complementary foods. Therefore, fermentation can be used as a nutrient improvement technique especially in formulations targeting protein malnutrition.

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