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FORTIFYING MUFFINS WITH PSYLLIUM HUSK FIBRE, OAT FIBER AND BARLEY FIBRE TO IMPROVE QUALITY AND SHELF LIFE

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ABSTRACT

Muffins were prepared after incorporation of 5, 10, 15 and 20 per cent fibers viz. oat, psyllium and barley and were stored under ambient and refrigeration conditions. A significant increase in weight of muffins was observed with increasing level of fibers incorporation for muffin making. Muffins incorporated with 10 per cent oat, 10 per cent psyllium and 15 per cent barley fibres had more sensory score than the control muffins. A significant decrease in compression force was observed with increasing levels of fibers in flour for muffins making. A significant decrease in the moisture contents of the muffins was observed in both refrigerated and ambient conditions with increasing time. The sample became dry and finally became unacceptable. Water activity of muffins was found higher in the refrigeration condition but there was a non significant variation in water activity of muffins stored at refrigeration conditions. However, it varied significantly at ambient conditions. Free fatty acid content (% oleic acid) also increased with increasing storage period but product did not develop off flavors under refrigeration storage. During storage Total Plate Count and Yeast and Mold Count (cfu per g) of the muffins increased significantly. The growth was more and became visible early in control muffins stored under ambient conditions than fiber incorporated muffins. The overall acceptability of the muffins decreased under storage since they became hard due to loss of moisture and also due to free fatty acid and peroxide productions.

1. Introduction

Fiber has many health benefits. Eating soluble fiber had been shown to reduce the risk of developing heart disease by reducing cholesterol levels. Eating insoluble fiber had been shown to reduce the risk of developing constipation, colitis, colon cancer and even haemorrhoids. Diabetics on high-fiber diets need much less insulin. Because fiber fills you up without adding calories, it could help prevent and treat obesity.

Oats can reduce blood cholesterol levels because of their soluble fibre content. The enriched oat contains 4g soluble fibre per 100g. Psyllium is mainly used as a dietary fiber, which is not absorbed by the small intestine. The purely mechanical action of psyllium mucilage absorbs excess water while stimulating normal bowel elimination. Although its main use has been as a laxative, it is more appropriately termed a true dietary fiber and as such can help to reduce the symptoms of both constipation and mild diarrhea. The laxative properties of psyllium are attributed to the fiber absorbing water and subsequently softening the stool. Barley fibre contains more than twice the amount of insoluble and soluble fibre found in

wheat or oats, as well as resistant starch, which helps promote healthy digestive bacteria. The products made with barley fibre such as breakfast cereals, muffins and breads have a low glycemic index (GI) and strong bowel health attributes (Dougherty et al., 1988).

The present day consumer looks for new bakery products, better appeal, taste and convenience from bakery foods. With a population of 1 billion plus, India has the largest middle-income consumers, who demand varieties in food, clothing, transport and improved living standards, also wish to eat out. Opportunities, in abundance, exist in bakery, cafes and restaurants and who fast food enters which is another fastest growing sector in India. This is a new challenge to baker and needs to update information, technology, products and services to meet the changing needs of the Indian consumer.

Preservation in bakery means the retardation of spoilage including the texture staling. Incorporation of dietary fibre obtained from cereals, fruits and vegetables could be very useful to improve the texture and prolong the shelf life without staling. Apart from increase in the shelf life, the dietary fibre has a number of nutritional advantages. It is well established that it has hypocholesteromic/ hypolipidemic effects and consumption of high fibre food items is very useful for the persons suffering from atherosclerosis, constipation, obesity and diabetes etc.

Keeping these points in mind, the present study was planned with the objectives to optimize the level of fibres in muffins to find out the best level on the basis of quality, to find the overall acceptability of the muffins on the basis of sensory evaluation by panelists and to study the shelf life of muffins prepared after incorporation of fibres in suitable packaging material.

2. Materials and methods

2.1. Raw materials

Flour, sugar, fibres (oat, psyllium and barley), salt, yeast and other ingredients for muffins preparation were procured from local market.

2.2. Chemical analysis of flour

Chemical characteristics of flour were analyzed using standard procedures (AACC, 2000).

2.3 Treatments

Muffins prepared after incorporation of fibre such as barley, psyllium and oat at levels of 0-20 per cent.

2.4. Product preparation

Muffins were prepared according to standard procedures (AACC, 2000) with slight modifications.

2.5. Sensory evaluation

Product prepared was evaluated for sensory properties by panel of semi trained judges (Larmond, 1970).

2.6. Calorific value

Calorific value determined by using Bomb Calorimeter, Parr Calorimeter Assembly-6100 (Parr Instrument Company, Moline, Ilinois 61265, U.S.A).

2.7. Shelf life

After preparation, the control and fibre enriched muffins were packed in Linear Low density Polyethylene (LLDPE) and were stored for 30 days at ambient (30±1ºC) and refrigerated (4-6ºC) conditions. Periodic analysis for moisture, water activity and free fatty acid were carried out to asses the shelf life.

2.8. Observations

Flour quality, product quality, organoleptic quality and shelf life of product were observed.

2.9. Statistical analysis

Data obtained was analyzed statistically using techniques of analysis of variance (ANOVA) (Singh et al., 1991).

3. Results and discussions

3.1. Flour characteristics

The flour had 11.99 per cent protein, 0.85 per cent fat, 0.44 per cent ash, 0.035 per cent free fatty acid (as % oleic acid), 28.92 per cent wet gluten, 8.42 per cent dry gluten, 203.75 mg maltose/10 g flour total sugars and 30.26 mg maltose/10 g of reducing sugars.

3.2. Pasting properties of flour

The effect of incorporation of oat, psyllium and barley fibres at 5, 10, 15 and 20 per cent level on pasting properties of flour for muffin making is discussed in Table 1. Pasting temperatures for the flour fibre combination varied significantly. The pasting temperature increased with the addition of fibres in flour and with the increasing levels. Pasting temperature for control was 93.2°C which increased to 94.60°C at 20 per cent incorporation of oat fibre. Peak viscosity might reach at the equilibrium point between swelling and polymer leaching which caused increase in viscosity. Peak viscosity of oat fibre was 1855 cP at 5 percent level of incorporation of increased to 2683 cP at 20 per cent level of incorporation. Similar, results were observed for psyllium and barley fibres i.e. it increased with increased level of fibre incorporation.

Hold viscosity for different level of addition of fibres (oat, psyllium and barley) varied significantly. There was no n significant variation in change of hold viscosity with the increased levels of fibre addition. With the addition of fibre increased the final viscosities, breakdown viscosity and setback viscosities as compared to control. Among the fibres, psyllium fibre exhibited maximum final viscosity at 20 per cent level of addition (6029 cP). Dengate and Meredith (1984) reported that peak viscosity was dependent on swelling, exudation and fragmentation of starch. On the other hand, breakdown viscosity was regarded as measure of degree of disintegration of starch granule or substances.

3.2.1. Baking and textural properties of muffins

The effect of incorporation of oat, psyllium and barley fibers on baking and textural properties of muffins is discussed in Table 2. Weight of muffins varied significantly for all fibre incorporation for muffins making. Weight of muffins increased significantly with the addition of fibers for muffins making. Among the fiber muffins maximum weight was found at 20 per cent psyllium fiber (48.94g) as compared to control which was 43.91g. Fibers had tendency to absorb more water, prevent the loss of water during baking process. So fibers decreased baking losses and resulted in muffins with higher weight, low volume and compact grain structure with soft texture.

No definite trend was observed in volume of muffins in reference to control but it varied significantly with the addition of fibre. Volume for oat fibre incorporation varied from 125cc for control to 123.33cc at 20 per cent level of incorporation (123.33cc). Volume for psyllium fibre incorporated muffins was found maximum at 10 per cent level of incorporation of psyllium fibre. No definite trend was observed in volume of muffins incorporated with 5, 10, 15 and 20 per cent barley fibre in reference to control but it was found maximum at 15 per cent level of barley fibre incorporation (129.67cc). No definite trend was observed in specific volume of muffins in reference to control, but it varied significantly with addition of oat and barley fibre. Specific volume of muffins decreased significantly with the increased level of psyllium fibre. Among the fibre maximum specific volume was observed at 15 per cent barley fibre incorporation. Decrease in volumes had been reported by several researchers (Burkit et al.,

1974). Bhatty (1993) reported that β-glucans contained in hulless barley might be undesirable in some food applications due to their high viscosities. Liangli et al. (2003) reported that psyllium fibre incorporation showed difficulty in handling of dough due to it's more gelling and water absorption qualities. Park et al. (1997) substituted 7:3 (w/w) mixtures of wheat fibre and psyllium fibre for preparation of bread.

Firmness decreased significantly with the addition of fiber in flour for muffins making. With the incorporation of fibre in flour for muffins making, firmness of muffins decreased with the increased level of fibre incorporation. Among the fibers minimum firmness was 1.66 kg at 20 per cent level of psyllium fibre followed by 15 per cent, 10 per cent and 5 per cent levels. The reason for increased softness on addition of fibers might have been due to increased water holding capacity of fibers.

Bread hardening effect of fibres have been subsequently reported (Pomeranz et al., 1977) and connected to the diluting gluten content of crumb structure disruption encompassing impairment in gas retention. Minimized dough stickiness and adhesiveness are suitable trends to fit textural requirements providing good muffins making performance. Stickiness value decreased with increasing fiber levels.

3.4. Sensory evaluation of muffins

The effect of incorporation of oat, psyllium and barley fibres at 5, 10, 15 and 20 per cent on the mean sensory panel scores of muffins is discussed in Table 3. Mean sensory panel score for muffins prepared after incorporation of oat (5, 10, 15 and 20 per cent) psyllium (5, 10, 15 and 20 per cent) and barley fibres (5, 10, 15 and 20 per cent) were evaluated by trained panel of judges on nine point hedonic scale (Table 3). Statistically significant variations were observed with regard to organoleptic quality such as appearance, texture, grain, flavor and overall acceptability, while score for the color of muffins varied not significantly.

No definite trend was observed for score given to appearance of muffins in reference to control but it varied significantly with addition of oat, psyllium and barley fibres. Maximum score for the appearance was given to muffins prepared after incorporation of 10 per cent oat, 10 per cent psyllium and 15 per cent barley fibres were 8.30, 8.45 and 8.45, respectively in the order.

Texture for the control sample was 7.18. No definite trend was observed for score given to texture of muffins in reference to control but it varied significantly with addition of oat (5, 10, 15 and 20 per cent) psyllium (5, 10, 15 and 20 per cent) and barley fibres (5, 10, 15 and 20 per cent) incorporation. Score for the grain structure of muffins prepared after incorporation of oat fibre at 5, 10, 15 and 20 per cent level were 7.85, 7.85, 8.15 and 7.75, respectively as compared to control muffin which was 7.48. Score for the grains of muffins prepared after incorporation of 10 per cent psyllium fibre was maximum (8.40) as compared to control which was 7.48. Score for the grain structure of muffins prepared after incorporation of barley fibre at 5, 10, 15 and 20 per cent level were 8.50, 8.20, 8.30 and 7.50 respectively (Figures 1 and 2).

No definite trends were observed for score given to flavor in reference to control but it varied significantly with the addition of psyllium and barley fibres at 5, 10, 15 and 20 per cent level of each. Score given to oat fiber muffins increased with the increased level of fiber addition from 7.70 for control to 8.05 at 20 per cent oat fibre incorporation but it varied non significantly in reference to control. Similarly, no definite trend was observed for score given to grain structure of muffins in reference to control but varied significantly with addition of oat, psyllium and barley fibers.

Significant variations were observed in overall acceptability of muffins added with oat, psyllium and barley fibers at 5, 10, 15 and 20 per cent level of incorporations. Maximum score were given to overall acceptability of

muffins at 10 per cent psyllium fiber incorporation (8.37) as compared to control which was 7.53. Similarly, maximum score for the overall acceptability of muffins were given at 15 per cent barley fiber incorporation (8.34) as compared to control which was 7.53. Oat fibre as increasing levels showed increased scoring by panelists for all parameters, regarding organoleptic quality. Maximum score were given at 15 per cent level of incorporation of oat fiber in muffins. Mckechnie (1983) reported that oat flour could be substituted as much as 30.0 per cent of the wheat fibre in bread. The 40 per cent oat flour supplemented bread had significantly softened texture than all other breads (Salehifar and Shahedi, 2001).

Color was given lower score for psyllium fibre incorporated muffins. This could have been because of appearance black speaks on dark brown background and these were increased with increased levels of incorporation of psyllium fibre. Similarly results were reported by Park et al. (1997) that fiber bread showed somewhat inferior crumb grain with an off flavor caused by small black speaks on dark background. Ahluwalia et al. (1995) reported that supplementary what flour with psyllium fiber up to 2 per cent level produced acceptable quality of bread.

3.5. Calorific value

Calorie content for control sample was 4.96 Kcal/g.Significant variations were found in calorie of muffins. Calorie content of muffins decreased with increased level of fiber. With the incorporation of oat fiber at 5, 10 15 and 20 per cent in flour for muffins making, calorie content decreased from 4.27 Kcal/g at 5 per cent level to 4.09 Kcal/g at 20 per cent level. With the incorporation of psyllium fiber at 5, 10 15 and 20 per cent in flour for muffins making, calorie content decreased from 4.02 Kcal/g at 5 per cent level to 3.81 Kcal/g at 20 per cent level of incorporation**.** With the incorporation of barley fiber at 5, 10 15 and 20 per cent in flour for muffins making, calorie content decreased from 4.47 Kcal/g at 5 per cent level to 4.03 Kcal/g at 20 per cent level of incorporation. Calorie reduction was found minimum at 5 per cent level of incorporation of barley fiber for muffin making (9.87 per cent). It was maximum at 20 per cent level of barley fiber incorporation for muffin making (18.75 per cent).

3.6. Shelf life study

Shelf life of muffins prepared after incorporation best level of fibers was studied. Muffins were analyzed for moisture, water activity, free fatty acid and overall acceptability under ambient temperature (30±1ºC) and refrigerated temperature (4-6ºC) conditions.

Muffins were packed in Linear Low Density Polyethylene (LLDPE) and stored under different temperature conditions and their moisture content; water activity, free fatty acid and overall acceptability were estimated for 35 days after 7 days interval.

3.6.1. Moisture content

The muffins stored under ambient conditions showed a higher rate of moisture loss than those stored under refrigerated conditions (Table 4). Higher moisture content was found in muffins prepared after incorporation of 15 per cent barley fiber as compared to control. Barley fiber had high capacity to absorb more water than other fiber sources. The muffins prepared after incorporation of 15 per cent barley fiber had maximum moisture content. Moisture retention property of fiber keeps muffins fresher for longer period of time (Forssell et al., 1998). Higher moisture retention in muffins is economical and also required to lengthen shelf life. Rogers et al. (1988) reported that moisture content was inversely proportional to the rate of firming. Hoseney and He (1990) concluded that moisture content significantly affected bread firming. Higher the moisture, slower the firming rate and the lower the final firmness.

Table 1. Effect of incorporation of fibres on the pasting properties of flour used for muffins making

Fibess	Level (%)	Parameters					
		Paste temperature (°C)	Peak viscosity (cP)	Hold viscosity (cP)	Final viscosity (cP)	Breakdown viscosity (cP)	Setback viscosity (cP)
Control	0	93.2	1657	981	1516	544	1139
Oat	5	93.90	1855	1178	2835	677	1657
	10	94.10	1956	1223	2991	733	1768
	15	94.30	2396	1456	3488	940	2032
	20	94.60	2683	1656	3815	1027	2159
Psyllium	5	89.50	1396	824	1716	572	892
	10	68.10	2716	1500	3307	1216	1807
	15	68.10	3278	1596	3175	1682	1579
	20	65.00	6363	1999	6029	3364	3030
Barley	5	94.70	2506	1344	3326	1982	1982
	10	94.30	2981	1572	3847	2275	2275
	15	93.60	3563	1854	4479	2645	2645
	20	93.10	3876	2003	4976	2973	2645
LSD ($p<0.05$)		0.17	0.14	0.93	0.10	0.93	2973

*LSD- Least significant difference

Table 2. Effect of incorporation of fibers on baking and textural properties of muffins

Fibres	Level (%)	Weight (g)	Volume (cc)	Specific volume (cc/g)	Compression force (Kg)
Control	0	43.91	125.00	2.84	7.39
Oat	5	44.46	123.33	2.77	4.70
	10	45.19	129.85	2.87	3.68
	15	43.61	125.00	2.86	4.47
	20	44.36	123.33	2.78	5.74
Psyllium	5	4368	120.00	2.74	1.78
	10	48.69	123.33	2.54	1.73
	15	47.74	111.66	2.34	1.71
	20	48.94	115.00	2.36	1.66
Barley	5	43.53	118.33	2.71	3.01
	10	45.81	126.20	2.75	2.29
	15	47.03	129.67	2.76	1.77
	20	44.36	116.66	2.63	1.81
LSD($p<0.05$)		4.04	6.00	0.22	0.26

*LSD- Least significant difference

Table 3. Effect of incorporation of fibers on the mean sensory panel scores (Max 9) of muffins

Fibers	Level (%)	Parameters					
		Color	Appearance	Texture	Grain	Flavor	Overall acceptability
Control	0	8.00	7.80	7.18	7.48	7.70	7.53
Oat	5	7.85	8.10	7.85	7.85	7.75	7.85
	10	7.75	8.30	7.85	7.85	7.95	7.95
	15	7.80	6.70	8.10	8.15	7.95	8.17
	20	7.85	7.80	8.00	7.75	8.05	7.95
Psyllium	5	7.95	7.85	8.10	8.10	8.35	8.01
	10	7.90	8.45	8.55	8.40	8.55	8.37
	15	8.45	8.10	8.45	8.10	8.40	8.30
	20	7.90	8.00	7.95	7.90	8.15	7.92
Barley	5	8.15	8.45	8.10	8.50	8.50	8.33
	10	8.10	8.40	8.60	8.20	8.30	8.26
	15	7.90	8.45	8.55	8.30	8.50	8.34
	20	7.50	7.70	7.80	7.50	7.20	7.36
LSD($p<0.05$)		NS	0.61	0.61	0.63	0.54	0.45

*LSD- Least significant difference
*NS – Non significant

Table 4. Effect of storage condition and period on moisture content (%) of muffins prepared by adding fibers packed in LLDPE

Days	LLDPE							
	Ambient ($30 \pm 10\ ^{O}C$)				Refrigerated ($4\text{-}6\ ^{O}C$)			
	F_1	F_2	F_3	F_4	F_1	F_2	F_3	F_4
0	22.97	24.49	23.03	24.74	22.57	23.21	21.29	20.23
7	21.63	24.15	22.64	23.36	22.16	22.88	21.67	20.88
14	20.86	21.35	21.08	21.83	20.61	23.01	22.19	21.78
21	19.20	20.60	19.41	20.03	21.90	23.45	22.55	21.98
28	17.49	18.38	17.03	19.63	22.10	23.97	23.45	22.44
35	ND	ND	ND	ND	23.51	24.35	23.67	23.36
LSD ($p<0.05$)	0.77				0.86			

LLDPE – Linear low density polyethylene,
LSD- Least significant difference,
ND – Not determined due to sample become unacceptable
F_1 – Control, F_2- 10% Oat fibre, F_3 - 10% Psyllium fibre , F_4 - 15% Barley fibre

Table 5. Effect of storage condition and period on water activity of muffins prepared by adding fibers packed in LLDPE

Days	LLDPE							
	Ambient (30 ± 10 ^{O}C)				Refrigerated (4-6^{O}C)			
	F_1	F_2	F_3	F_4	F_1	F_2	F_3	F_4
0	0.81	0.73	0.67	0.65	0.76	0.67	0.68	0.61
7	0.83	0.75	0.70	0.68	0.78	0.68	0.69	0.63
14	0.85	0.77	0.73	0.71	0.79	0.70	0.72	0.65
21	0.89	0.81	0.78	0.74	0.81	0.72	0.73	0.67
28	0.92	0.83	0.80	0.79	0.84	0.74	0.74	0.71
35	ND	ND	ND	ND	0.85	0.76	0.75	0.74
LSD ($p<0.05$)	0.019				0.013			

LLDPE – Linear low density polyethylene, LSD- Least significant difference, ND – Not determined due to sample become unacceptable, F_1 – Control, F_2- 10% Oat fibre, F_3 - 10% Psyllium fibre , F_4 - 15% Barley fibre

Table 6. Effect of storage condition and period on free fatty acid content (% oleic acid) of muffins prepared by adding fibers packed in LLDPE

Days	LLDPE							
	Ambient (30 ± 10^{O}C)				Refrigerated (4-6^{O}C)			
	F_1	F_2	F_3	F_4	F_1	F_2	F_3	F_4
0	0.54	0.53	0.54	0.47	0.55	0.49	0.53	0.46
7	0.60	0.60	0.64	0.56	0.65	0.55	0.59	0.49
14	0.70	0.65	0.71	0.63	0.71	0.60	0.65	0.54
21	0.77	0.70	0.76	0.67	0.76	0.65	0.70	0.61
28	0.85	0.80	0.81	0.73	0.77	0.74	0.73	0.69
35	ND	ND	ND	ND	0.85	0.79	0.78	0.74
LSD($p<0.05$)	0.038				0.048			

Table 7. Effect of storage condition and period on overall acceptability of muffins prepared by adding fibers packed in LLDPE

Days	LLDPE							
	Ambient (30 ± 10 ^{O}C)				Refrigerated (4-6^{O}C)			
	F_1	F_2	F_3	F_4	F_1	F_2	F_3	F_4
0	8.32	7.53	8.45	8.56	8.32	7.71	8.67	8.61
7	8.11	7.85	8.01	8.34	8.21	7.42	8.21	8.44
14	7.54	7.95	8.27	8.26	7.86	7.30	8.01	8.16
21	6.36	8.18	8.31	7.85	7.26	7.11	7.78	7.92
28	5.43	7.96	7.92	7.36	6.89	6.78	7.63	7.24
35	ND	ND	ND	ND	6.31	6.29	7.25	7.22
LSD ($p<0.05$)	0.17				0.27			

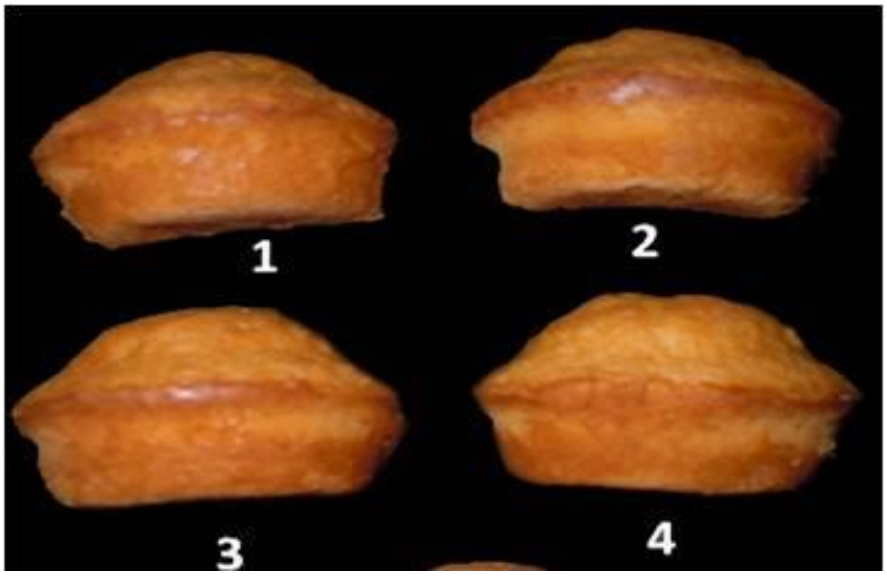

Figure1. Effect of incorporation of best level of fibres on baking quality of muffins
1 – Control, 2 – 10 % Oat fiber, 3 – 10 % Psyllium fiber , 4 –15 % Barley fiber

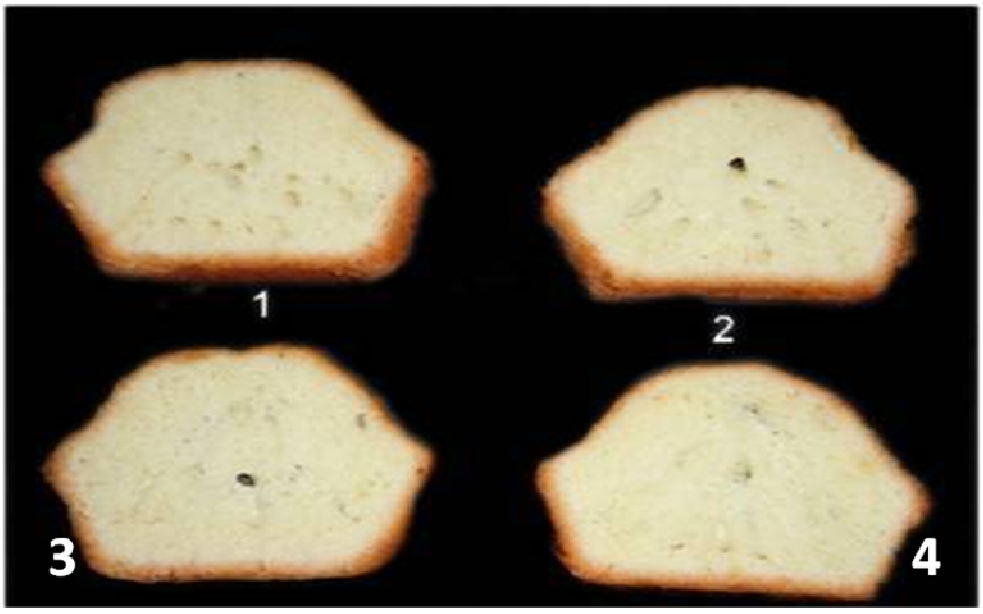

Figure 2. Effect of incorporation of best level of fibers on baking quality of muffins
1 – Control, 2 – 10 % Oat fiber, 3 – 10 % Psyllium fiber, 4 –15 % Barley fiber

3.6.2. Water activity

Statistically significant variations were observed in the moisture content of muffins prepared with fibers with respect to day of storage and temperature of storage (Table 5). Water acidity of muffins increased with increased storage period. Similar results were observed by Frazier (1978). Water activity found less for muffins prepared from fibers than control muffins. Increase in water activity of muffins prepared from fibers was less than that of control muffins. Barley fiber muffins had lower water activity (0.79) than muffins prepared from oat fibre (0.83) and psyllium fiber (0.80). Water activity of muffins varied significantly with respect to storage condition and period. At refrigeration storage, water activity increased with the storage time. Arya (1980) reported that storage at less than 0.57 a_w did not cause perceptible change in flavor for 24-52 days. Rossel et al. (2001) reported that the hydroxyl group of the fibre structure which allow more water interaction through bonding. Oat starch had higher water absorption than other cereals. Labuza et al. (1972) reported that reducing water activity below 0.7 prevent microbial spoilage.

3.6.3. Total plate count and yeast and mold growth

At refrigeration condition mold growth was not found up to 35 days of storage while mold growth on muffins prepared after incorporation of fibres found after 28 days at ambient temperature. Similar observation was reported by Breene et al. (1988).

3.6.4. Free fatty acid (as % oleic acid)

Statistically significant variations were observed in development of free fatty acids in the muffins stored under ambient conditions (Table 6). The development of free fatty acids was lower in case of muffins and it was found to be highest on the 28thday of storage. Muffins stored under ambient condition showed higher amount of free fatty acid than muffins stored under refrigeration conditions. Gain of moisture by the product promoted oxidation of fats. Similar results were obtained by Singh et al. (2000) who reported that free fatty acid content of all biscuits increased gradually with the increase in the storage period.

3.6.4. Overall acceptability

Statistically significant variations were observed in the overall acceptability of muffins stored under ambient and refrigeration conditions (Table 7). Overall acceptability of muffins decreased with increased storage period from 7th to 28th days for ambient storage and from 7th to 35th days for refrigeration storage due change in texture of muffins. Overall acceptability decreased more in muffins stored under ambient temperature than that for refrigeration temperature.

4. Conclusions

The effect of incorporation of oat, psyllium and barley fibers at 5, 10, 15 and 20 per cent on muffin making and sensory properties revealed the increased muffin weight and decreased specific volume. The overall acceptability scores were maximum for muffins prepared with psyllium fiber at 10 per cent level (8.37), followed by barley fiber at 15 per cent level (8.34) and oat fiber at 10 per cent level (7.95), in the order. With the increasing level of incorporation of fibers, there was definite improvement in the texture of muffins i.e. hardness decreased means muffins become softer. Significant variations in various parameters were observed during storage. A significant decrease in the moisture content of the muffins was observed in both refrigerated and ambient conditions with increasing time. The sample became dry and finally unacceptable. The overall acceptability of the muffins decreased with increasing storage time since they became hard due to loss of moisture and also due to development of free fatty acid. At refrigeration storage, water activity increased with the increased storage time. Free

fatty acid content (% oleic acid) was observed to be higher in the muffins stored at ambient condition than refrigeration condition. The storage period had significant effect on the overall acceptability of muffins stored in ambient and refrigeration conditions. The overall acceptability of muffins decreased with the increasing storage period. Muffins prepared after incorporation of fibres remained acceptable till 28 days at ambient condition and 35 days when stored under refrigerated condition in LLDPE but sensory properties altered after 35 days. Shelf life of control muffin was 21 days which increased to 35 days by incorporation of fibres in muffin making. The increase shelf life will be helpful for the bakery industry which is growing at the significant rate of 40 per cent annually.

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EXTRACTION OPTIMIZATION OF PECTIN FROM FRESH CITRUS PEEL WITH RESPONSE SURFACE METHODOLOGY

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ABSTRACT

This study aims at optimizing the extraction process parameters of natural pectin from citrus peel, a kind of waste resource in orange production industry, containing high content of pectin. Single factor test and surface response methodology were employed to optimize the values of temperature, time, ratio of solid and liquid, and pH in the pectin extraction. The result indicates that optimized values of extraction time, extraction temperature, pH, and ratio of solid and liquid are 1.5 h, 90 ^{o}C, 2, and 1:35, respectively. The second degree polynomial equation for this surface response model could be expressed as follows:

$$\text{Yield rate}\,(\%) = 0.85 + 0.086\,A + 0.06\,B - 0.23\,C - 0.08\,AB + 0.012\,AC - 0.045\,BC - 0.22\,A^2 - 0.028\,B^2 - 0.081\,C^2$$

The high model *F* value and low *P* value, which are 22.59 and 0.0002, respectively, indicate that this surface response model is highly significant.

1. Introduction

Pectin, also known as pectic polysaccharide, exists in many kinds of vegetables and fruits (Chacko and Estherlydia, 2013). The health function of pectin has been realized by more and more people. The addition of pectin in food, such as bread and steamed bread, could not only improve the taste and property of food, but also enhance the nutrient value (He and Lu, 2014). It was also reported that pectin could prevent heart diseases and reduce the risk of high blood intension (Sanders, 1998, Willats et al., 2006). Furthermore, pectin is also the raw material for the production of many jells and fruit sauces. With the development of food industry, the demand for pectin is increasing rapidly (Willats et al., 2006). Therefore, how to produce natural pectin with high quality at low cost has become a hot topic.

Orange is used in the production of orange juice and sauce in food processing industry. However, the waste, including orange pomace and orange peel, of food industry has become a serious problem because inappropriate treatment of these wastes will cause serious environmental pollution (Denisa et al., 2014). It is reported that citrus peel contains about 30% natural pectin, which is much higher than the pectin content in other natural materials, such as apple pomace and carrot (Syed et al., 2011). Furthermore, the production cost of pectin could be reduced if the citrus peel could be used as a source of pectin in the production. It was reported that the pectin extracted from citrus peel contains around 15% soluble dietary

fiber which could prevent the colorectal cancer (Sanders, 1998, Yapo et al., 2007). Therefore, the pectin extracted from citrus peel is much more valuable than that from other resources.

This study aims at improving the extraction yield of pectin from orange peels with acid solution at low cost. To optimize the extraction process parameters, surface response method was employed to evaluate the impact of four factors, ratio of solid and liquid, extraction temperature, extraction time, and pH value, on the yield of pectin.

2. Materials and methods

2.1. Materials and chemicals

The citrus peel was obtained from oranges purchased from local market. The fresh citrus peel was stored at 4^{o}C in dark until use. Concentrated sulfuric acid (98%) was purchased from Sigma-Aldrich Co. LLC.

2.2. Extraction process

There were five main steps in the extraction process: 1. Cut citrus peel into small pieces (1.5~2 cm^2); 2. Heated the citrus peel sample at 90^{o}C for 10 min to inactivate the enzyme; 3. Washed the fresh citrus peel with stilled water; 4. Weighted about 4.0 g fresh citrus peel for the extraction; 5. Filtered the citrus peel and extracted the pectin with the water bath instrument under certain conditions.

To determine the content of pectin extracted from citrus peel, the absorbance at 531 nm by a spectrophotometer was read.

2.3. Single factor test

The extraction temperature, extraction time, and pH value were set as 80^{o}C, 1.5 h, and 2, respectively, in the evaluation of effect of ratio of solid and liquid. The yield rates of pectin when the ratios of solid and liquid were 1:20, 1:25, 1:30, 1:35, and 1:40 were compared. The optimized value of ratio of solid and liquid was determined according to the yield rate of pectin.

In the evaluation of effect of extraction temperature, the extraction time and pH value were set as 1 h and 2, respectively. The ratio of solid and liquid was set as the optimized value. The yield rates when the extraction temperatures were 70 ^{o}C, 80 ^{o}C, 90 ^{o}C, and 100 ^{o}C were compared. The optimized value of extraction temperature was determined according to the yield rate of pectin.

In the evaluation of effect of extraction time, the pH value was set as 2 and the extraction temperature and ratio of solid and liquid were set as the optimized value. The yield rates when the extraction time was 0.5 h, 1 h, 1.5 h, 2 h, and 2.5 h were evaluated. The optimized value of extraction time was determined according to the yield rate of pectin.

The extraction temperature, extraction time, and ratio of solid and liquid were set as the optimized values in the evaluation of effects of pH value. The yield rates when the pH values were 1.0, 1.5, 2.0, 2.5, and 3.0 were compared. The optimized value of pH value was determined according to the yield rate of pectin.

2.4. Box-Behnken Design (BBD)

Box-Behnken experiment was designed according to the result of single factor test. In the Box-Behnken experiment, three factors, including extraction temperature, extraction time and pH value, were analyzed. Response surface methodology was employed to determine optimize the independent variables relating with the yield rate of pectin from citrus peel. A second degree polynomial equation (Eq. 1) was used to mathematically describe the extraction process and estimate the response of the dependent variable.

$$Y = a_0 + \sum_{i=1}^{n} a_i x + \sum_{i=1}^{n} a_{ii} x^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} a_{ij} x_i x_j \quad (1)$$

where: Y is the response variables; x_i and x_j are the levels of the independent variables; a_0 is a

constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interactive coefficients, respectively.

We used the software of Design Expert to analyze the goodness-of-fit of the regression model and the significance of parameter estimates (Kim et al., 2004, Qiao et al., 2009).

3. Results and discussions

3.1. Result of single factor test

Impact of different ratios of solid and liquid on the yield rate of pectin from citrus peel was shown in Figure 1(a). It indicated that the yield rate increased with the decrease of ratio of solid and liquid. As the ratio decreased from 1:20 to 1:35, the yield rate of pectin increased by 85.71%. After the ratio of solid and liquid reaching 1:35, decreasing the ratio could not increase the yield rate of pectin. In the pectin production industry, vacuum condensation technology is employed to condensate the pectin in extraction solvent. Therefore, decreasing the ratio of solid and liquid could increase the production cost of pectin although it could improve the yield rate. Therefore, in the large scale production of pectin from citrus peel, the relationship between extraction cost and yield rate of pectin should be balanced according to the actual situations. In this study, to get the high yield rate, the ratio of solid and liquid was set as 1:35.

Figure 1(b), which reflects the impact of extraction temperature on the yield rate of pectin, indicates that the yield rate of pectin increased with the increase of extraction temperature when the temperature was below 90^{o}C. The reason is that higher temperature in certain range could accelerate the reaction rate and change the equilibrium of reaction. However, higher extraction (above 90^{o}C) temperature decreased the yield rate. The main reason is that high temperature could cause the degradation of galacturonic acid in citrus peel and finally reduce the yield rate of pectin. In large scale production of pectin, high extraction temperature could not only reduce the yield rate, but also cause the energy waste. Therefore, the optimization of extraction temperature is significant to the large scale production.

Effect of extraction time on the yield rate of pectin (Figure 1(c)) indicates that the yield rate of pectin increased with the extension of extraction time. The highest yield rate was 0.49% when the extraction time reached 1.5 h. When the extraction time was extended to 2.5 h, the yield rate decreased a little because of the degradation of galacturonic acid exposed to high temperature condition. Therefore, the optimized value of extraction time is 1.5 h.

Figure 1(d), which reflects the effect of pH value on the yield rate of pectin, shows that with the increase of pH value the yield rate of pectin decreased seriously. The main principle of acid extraction is using acids to destroy the connection between pectin and other polysaccharide molecules, and isolate the pectin. Increasing the pH value will reduce the acid concentration and negatively impact the isolation of pectin extraction.

Depending on the single factor test, the optimized values of extraction temperature, extraction time, ratio of solid and liquid, and pH were 90 ^{o}C, 1.5 h, 1:35, and 2, respectively.

3.2. Result of Box-Behnken experiment

The factors and levels in the Box-Behnken experimental design, the result of Box-Behnken experiment and analysis of variance are shown in Table 1, Table 2 and Table 3, respectively. Table 2 indicated that the yield rate of pectin reached the highest value (1.13%) when the extraction temperature, time and pH value were 90^{o}C, 2 h, and 1.0, respectively. The second degree polynomial equation which reflects the relationship between yield rate of pectin and three independent variables is shown as Eq.2:

$$\text{Yield rate}\,(\%) = 0.85 + 0.086\,A + 0.06\,B - 0.23\,C - 0.08\,AB + 0.012\,AC - 0.045\,BC - 0.22\,A^2 - 0.028\,B^2 - 0.081\,C^2 \quad (2)$$

Table 1. Design of Box-Behnken experiment

Factor	Levels		
	-1	0	1
A: Extraction temperature (^{o}C)	80	90	100
B: Extraction time (h)	1.0	1.5	2.0
C: pH value	1	1.5	2

Table 2. Box-Behnken experimental design matrix and corresponding experimental results

No.	A	B	C	Yield rate of pectin (%)
1	0	0	0	0.85
2	0	-1	1	0.45
3	0	-1	-1	0.89
4	0	0	0	0.91
5	0	1	1	0.51
6	0	1	-1	1.13
7	1	-1	0	0.76
8	0	0	0	0.87
9	1	1	0	0.69
10	1	0	-1	0.79
11	0	0	0	0.79
12	1	0	1	0.42
13	-1	-1	0	0.36
14	0	0	0	0.85
15	-1	1	0	0.61
16	-1	0	-1	0.71
17	-1	0	1	0.29

Table 3. Analysis of variance

Source	Sum of Squares	Degree of freedom	Mean Square	*F* Value	p-value Prob > F	Significance
Model	0.8002	9	0.0889	22.5958	0.0002	**
A-Extraction temperature	0.0595	1	0.0595	15.1239	0.0060	**
B-Extraction time	0.0288	1	0.0288	7.3189	0.0304	*
C-pH value	0.4278	1	0.4278	108.7198	< 0.0001	**
AB	0.0256	1	0.0256	6.5057	0.0381	*
AC	0.0006	1	0.0006	0.1588	0.7021	
BC	0.0081	1	0.0081	2.0584	0.1945	
A^2	0.2052	1	0.2052	52.1426	0.0002	**
B^2	0.0034	1	0.0034	0.8539	0.3862	
C^2	0.0275	1	0.0275	6.9771	0.0334	*
Residual	0.0275	7	0.0039			
Lack of Fit	0.0200	3	0.0067	3.5505	0.1263	
Pure Error	0.0075	4	0.0019			
Cor Total	0.8278	16				

* Significant ($p<0.05$); ** Extremely significant ($p<0.01$)

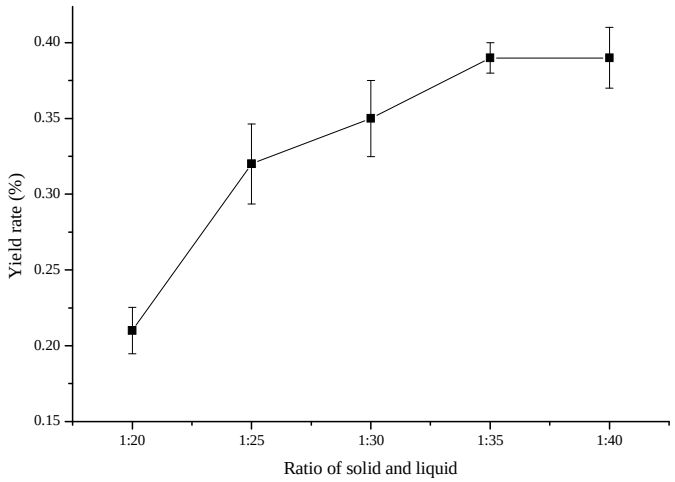

Figure 1(a). Impact of ratio of solid and liquid on yield rate

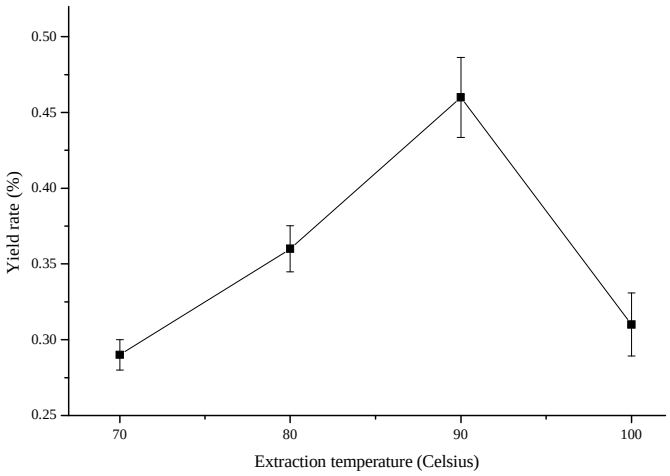

Figure 1(b). Impact of extraction temperature on yield rate

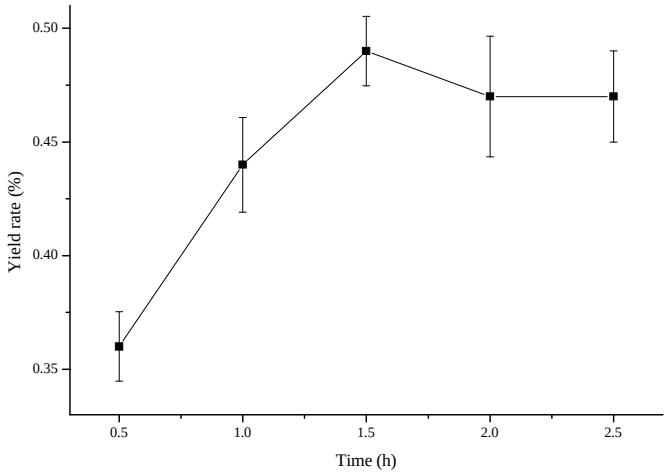

Figure 1(c). Impact of extraction time on yield rate

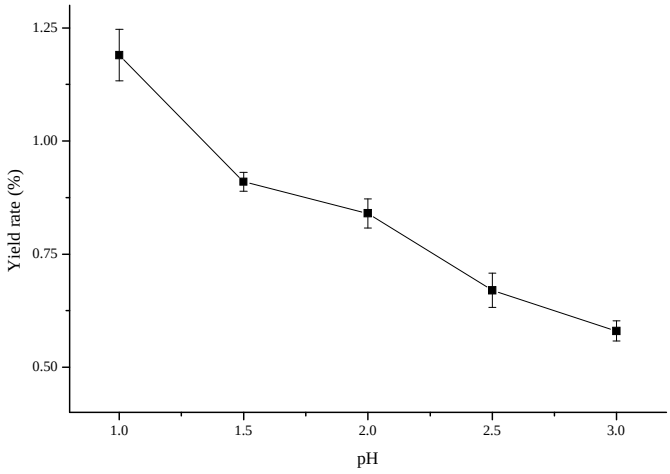

Figure 1(d). Impact of pH value on yield rate

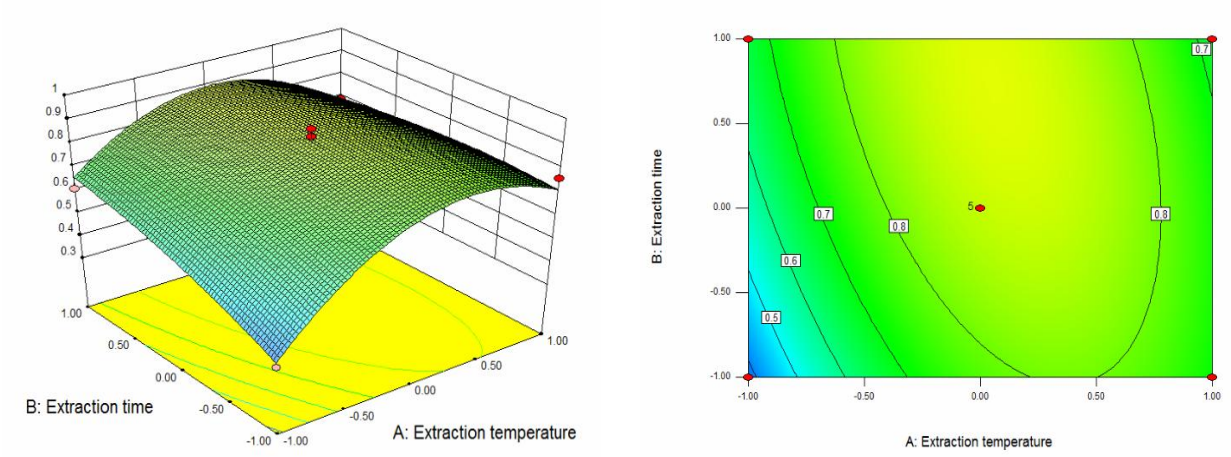

Figure 2. Response surface and contour plot for the effect of extraction temperature and time on the yield rate of pectin

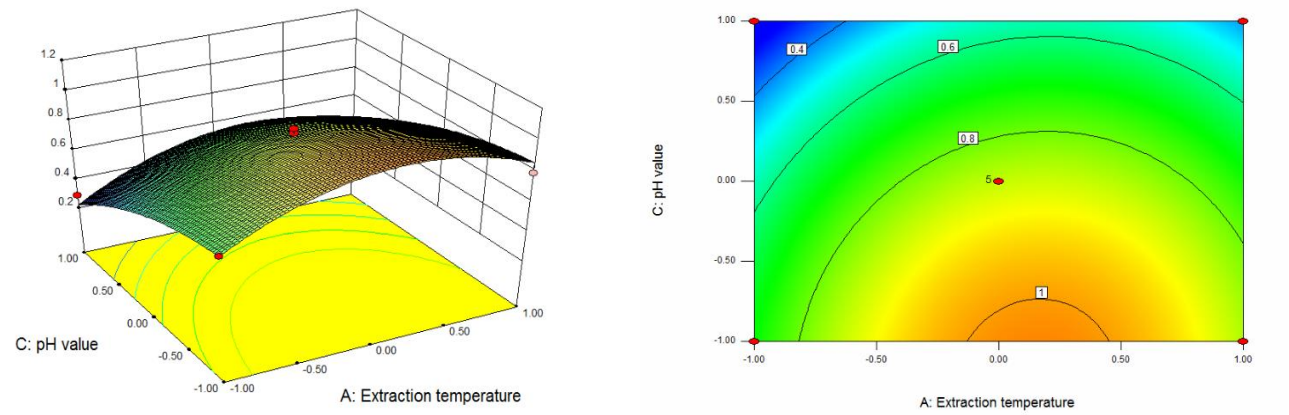

Figure 3. Response surface and contour plot for the effect of extraction temperature and pH value on the yield rate of pectin

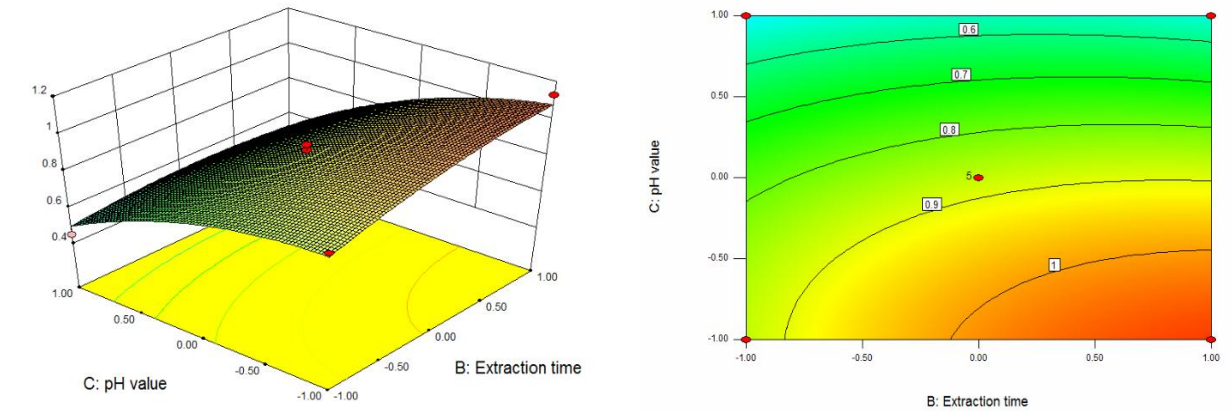

Figure 4. Response surface and contour plot for the effect of extraction time and pH value on the yield rate of pectin

Regression analysis was performed to fit the response function. The Model F-value of 22.59 implies the model is significant. There is only a 0.02% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.050 indicate model terms are significant. In this case, A, B, C, AB, A^2, and C^2 are significant model terms. The "Lack of Fit F-value" of 3.55 implies the Lack of Fit is not significant. There is a 12.63% chance that a "Lack of Fit F-value" this large could occur due to noise.

3.3. Response surface plot and optimized extraction process

Figure 2 presents the response surface and contour plot for the effect of extraction temperature and time on the yield rate of pectin. The yield rate increased with the increase of extraction temperature and extraction time in certain range. However, the result indicated that much high temperature and long extraction time could cause the degradation of pectin in the extraction process. The ellipse shaped curve in the contour plot picture indicated that there was significant interaction between extraction temperature and extraction time in this model.

Figure 3 presents the response surface and contour plot for the effect of extraction temperature and pH value on the yield rate of pectin. The increase of extraction temperature and decrease of pH value could improve the yield rate of pectin. However, between extraction temperature and pH value, there was no significant interaction.

Figure 4 presented the response surface and contour plot for the effect of extraction time and pH value on the yield rate of pectin. With the increase of extraction time and decrease of pH value, the yield rate if pectin increased. There was no significant interaction between extraction time and pH value.

In large scale production of pectin, the selection of optimum extraction parameters depends on various factors, such as production cost, limitation of time, pectin property and so on. For instance, if the time is limited and the working load is heavy in the pectin production, the temperature should be improved to reduce the extraction time although high temperature costs high energy. In addition, the adjustment of pH value may negatively impact the property of natural pectin. So to protect the quality of pectin in the extraction process, the pH value should not be too low although low pH value could improve the yield rate of pectin.

4. Conclusions

This study indicated that extraction temperature, extraction time, pH value and ratio of solid and liquid could impact the yield rate of pectin from citrus peel. The surface response analysis indicated that the yield rate of pectin from citrus peel could reach 1.13% at the optimum conditions. The high model *F* value and low *P* value, which are 22.59 and 0.0002, respectively, indicate that this surface response model is highly significant. In the large scale production of pectin, not only yield rate, but also many other factors, such as energy cost, pectin property, limitation of time and so on, should be taken into consideration. Therefore, in the production, the optimum conditions described in this study should be modified according to the actual situations.

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ANTIFUNGAL ACTIVITY OF GINGER AND CINNAMON LEAF ESSENTIAL OILS ON MANGO ANTHRACNOSE DISEASE CAUSING FUNGI (*C. gloeosporioides)*

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ABSTRACT

Mango (*Mangifera indica* L) is an important fruit crop in tropical and subtropical countries of the world and mango anthracnose disease is one of the major constraints to production and productivity of mango fruit world wide as well as in Ethiopia. Now a day, researchers are concentrating on the application of essential oils in the post-harvest preservation of the mango fruits due to their organic, safe and effective controlling nature. In such studies determination of the effective concentration of essential oil for the effective anti-microbial nature against the pathogenic fungi is very important. The objective of this study was to investigate the effect of ginger and cinnamon essential oils concentrations on mango anthracnose disease causing fungi. In the present study anthracnose affected mango fruits were collected from the field and the pathogenic fungi was isolated, identified and purified scientifically. Further, in-vitro studies were conducted with the three different concentration levels of each type of essential oils, 0.025, 0.050, 0.075% cinnamons and 0.15, 0.30, and 0.45% ginger and the control (distilled water). In the study we successfully isolated the responsible fungi for the anthracnose disease. The cinnamons and gingers essential oils at 0.075% and 0.045% respectively, were found to be highly effective on the fungal pathogen causing anthracnose disease on mango and can be recommended for the post harvest treatment of mango.

1. Introduction

Mango (*Mangifera indica* L.) is one of the world's most important and esteemed fruits of the tropical and subtropical countries and is cultivated extensively as a commercial fruit crop in India, China, Indonesia, Thailand and Mexico. By virtue of its wide range, delicious taste, super flavour, very high nutritive and medicinal value as well as great religio-historical significance, it is called the "King of the fruits" (Lakshmi and al., 2011). Mango is the most important fruit and it is the second in area coverage after banana in south-western region of Ethiopia (CSA, 2013). Various biotic and abiotic stresses cause immense loss to mango crop throughout the world**.** Post-harvest diseases are among those biotic stresses threatening mango fruit along the mango value chain. The most common diseases of mangoes are anthracnose (*Colletotrichum gloeosporioides*). Anthracnose is presently recognized as the most important field and

post-harvest disease of mango worldwide (Ploetz and Prakask, 1997). It is the major disease in all the countries where mangoes are grown, especially where high humidity prevails during the cropping season. The post-harvest phase is the most damaging and economically significant worldwide for disease. Antibiotics, oils, hot air treatment, hot water treatment, refrigerated storage and gas storage, copper fungicides, borax, aminoathiozol, amino pyridine, thiourea, dithiocarbamates, agrimycin, aureofungin, nystain, grisofulvin heated benomyl, oil and wax emulsion have been suggest as pre-harvest and post-harvest dip/spray for the prevention of anthracnose in mangoes (Panhwar, 2006)**.**

Essential oils are natural volatiles, limped and rarely colored, lipid soluble and soluble in organic solvents with generally lower density than water. Essential oils are complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites. In nature, essential oils play an important role in the protection of the plants as antibacterial, antiviral, antifungal, and insecticides. Compositionally, Essential oils contain alcohols, aldehydes, ketones, phenols, esters, ethers, and terpenes in varying proportions (Sharma and Pongener, 2010).

Anti-fungal tests in *in-vitro* conditions of several essential oils and their composition have been documented (Dafera et al., 2003; Linde et al., 2010; Soylu et al., 2010; Marei et al., 2012). Essential oils can be synthesized by all plant parts, i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretor cells, cavities, canals, epidemic cells or glandular. Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to others components present in trace amounts. The major components determine the biological properties of the essential oils. The components include two groups of distinct biosynthetical origin (Croteau et al., 2000; Betts, 2001; Pichersky et al., 2006). The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight. Rhizomes of ginger (*Zingiber officinale*) are one of the most important and oldest spices.

The ginger essential oil is characterized by warm, spicy, and woody notes, with slight lemony notes. The oil is pale yellow, low viscosity liquids, with refractive indices of 1.4884 to 1.4918, the densities of 0.883 to 0.877, and optical rotations of – 33.9 to – 39. This essential oil is dominated by α-zingiberene with low cucurmene content and traces amounts of neural and geranial. Whereas, Essential oil extracted from cinnamon leaves is yellow, with warm-spicy notes characterized simply as "cinnamon notes". The refractive index (1.5817 – 1.5909) and density (1.019 – 1.026) of the commercial samples were closer to pure cinnamic aldehyde, reflecting the higher levels of cinnamic aldehydes (Janick and Whipkey, 2002).

The mechanisms of the antimicrobial activity of essential oils are poorly understood for us, but according to previous reports, it seems that damage to cell walls and membrane structure and function is as antimicrobial action of essential oils (Rattanapitigorn et al., 2006, Cox et al., 2008). Essential oils from various plant species affect and arrest fungal development *in vitro* and *in vivo* in various horticultural commodities (Bosquez-Molinaa et al., 2010). These are used as natural fungicides to control pathogenic fungi and thus reduce the dependence on the synthetic fungicides (Fawzi et al., 2009). Also, due to their bactericidal and fungicidal properties, pharmaceutical and food uses are more widespread as alternatives to synthetic chemical products to protect the ecological equilibrium. Earlier studies reported relatively less on the effect of essential oils on the Mango Anthracnose disease causing fungi (*C. gloeosporioides*) in Ethiopia. So the present study was conducted to isolate, identify and purify the Mango Anthracnose disease causing fungi and to determine the effect of different concentrations of cinnamon and ginger

essential oils on the pathogen as the major objectives. It will be further useful to recommend the researcher to use the best concentration of these essential oils for the post harvest storage studies of mango fruits.

2. Materials and methods

2.1. Samples Collection

Mango (*Mangifera indica* L.) variety of apple mango was obtained from Jimma Agriculture Research Centre (JARC) and cinnamon leaf (*Cinnamomum zeylanicum)* and the rhizome of ginger (*Zingiber officinale*) were obtained from Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) garden and purchased from Bebeka Coffee Estate, Share Company, Ethiopia, respectively.

2.2. Extraction of essential oils from ginger and cinnamon

Three kilograms of each ginger rhizome and cinnamon leaves were collected and its size was reduced with grinder. Then they were blended separately in water until over flow in the flask. Extraction of essential oil was done using Clevenger Apparatus by hydrogen extraction method. After extraction the oils were collected and stored in refrigerator at 4ºC until use.

2.3. Media preparation

For *in vivo* studies Potato Dextrose Agar (PDA) medium was used, which is the common medium for isolation of different fungal species. Potato dextrose agar was prepared by dissolving commercially formulated powder PDA. The Potato dextrose agar powder was mixed with distilled sterilized water in 2000 mL flask at the ratio of 39 g powder: 1L distilled sterilized water and heated until melting. The mixtures were boiled by continuously stirring with a magnetic stirrer for about 15 minutes to completely dissolve the agar. The solution was then autoclaved at 121°C for 15 minutes to sterilize the media. The liquid media was placed in safety cabinet and allowed to cool to about 45°C. Then Streptomycin sulphate powder was added to the PDA media at the rate of l gm per Litre to avoid bacterial contamination and the media were poured into sterilized Petridish. Plates were then allowed to cool & solidified completely and preserved before inoculation of cultures.

2.4. Isolation, identification and purification of fungal pathogen

To isolate *C. gloeosporioides,* infected mango fruits were selected (Fig. 1 (A)). Five centimetre plugs of mango tissue were disinfected with 1% sodium hypochlorite and placed at the centre of a Petri dish containing potato dextrose agar. After 7 days of incubation period, the fungal culture was sub cultured (pure culture preparation) and confirmed to be C. *gloeosporioides* according to established procedures by Burnett and Hunter (Barnett and Hunter, 1972).

2.5. Pathogenicity test

To do pathogenecity test the green mature apple mango variety fruits were washed with tap water; sterilized with 2% sodium hypochlorite and rinsed with distilled water. Then the spore suspension of $10^6 ml^{-1}$ concentration was sprayed on it and incubated for about five days at room temperature. Purification and identification was done from inoculated mango fruit and it was verified to be the same result.

2.6.Spore suspension preparation for pathogenicity test

Colletotrichum gloeosporioides identified and preserved was cultured for about two weeks on PDA at 25°C to activate the pathogen. Spores were harvested by adding 5–7 ml of sterile, de- ionized water to the Petri dish. The spores were then rubbed with a sterile glass rod to make spores free from the PDA medium, and the spore suspension was passed through two layers of cheese cloth. The suspension was diluted with this sterilized and de-ionized water to obtain the spore concentrations (10^6 spores

mL^{-1}) with a haemocytometer (Abd-All and Haggag, 2013). Then the spore suspension prepared was preserved for about 5-10 minutes in the safety cabinet until used for sprayed on mango fruits.

2.7. Bioassay with essential oils against mycelia *C. gloeosporioides* - determination of the minimum inhibition concentration (MIC) value

Both ginger and cinnamon essential oils were individually evaluated for their fungal toxic activity against *Colletotrichum gloeosporioides* by adopting food poisoning technique into a petridish. For each fungal isolate, a conidial spore suspension of 10^6 spore's ml^{-1} was prepared and petridishes were inoculated with needle in the centre. The PDA medium was mixed with appropriate concentration of essential oil. The radial growth was measured with calliper and data was taken every 24hrs intervals after incubated at 25 ±2°C and 85% RH in triplicate. The MIC was determined as the lowest concentration of oil which results no growth of the inoculums on all Petridish. MIC was defined as the lowest oil concentration showing no visible growth after incubation time (Verma et al., 2011).

3. Results and discussions

3.1.Cultural characteristics of *Colletotrichum gloeosporioides* isolates

Cultural characteristics of purified isolates showed that the colony diameter of *C. gloeosporioides* reached up to 90 mm on PDA after 7 days at 25°C (Fig. 1 (B)). The colour of the mycelia appeared like white from the front side (Fig. 1(C)) and yellowish on the back side (Fig. 1(D)) when observed seven days after incubation. The morphological characteristics observed under microscope showed that conidia shape was cylindrical and had brown colour (Fig. 1(E)). This result is in line with the finding of reported study by Tasiwal (2008).

3.2. Pathogenicity of *C. gloeosporioides* on mango fruit

During pathogenicity test in five days of incubation the symptom of *C. gloeosporioides* observed was black spots and sunken lesions (Fig. 2). In order to confirm the observed symptom, isolation, purification and identification was done from inoculated mango fruit and it was verified to be the same result. Similarly, the colour of the mycelia appeared white from the front side and yellowish on the back side when observed seven days after incubation. The morphological characteristics observed under microscope showed that conidia shape was cylindrical and had brown colour. Hence, it was confirmed that the pathogen causing mango anthracnose in the study area is *C. gloeosporioides.* Result of pathogencity test also confirmed that *C. gloeosporioides* is the virulent causative agent of anthracnose in mango.

3.3. Minimum inhibition concentration of essential oils against *C. gloeosporioides*

Analysis of variance showed that mycelia growth of all ginger and cinnamon essential oil concentrations significantly ($P < 0.05$) different from control during the 8 day's incubation periods (Table 1). This means essential oils have effect on mycelia growth inhibition. Treatments with both cinnamon and ginger essential oil showed high anti-fungal effects on the mycelia growth of tested fungi even at lower concentrations. The mycelia growth inhibition was directly related to the concentrations and type of essential oil (Fig. 3). The findings of the present study are in agreement with Palhano et al (2004) who reported increased inhibitory effects of citrus and lemongrass crude oil on spore germination of *C. gloeosporioides* of mango fruit with increasing oil concentrations. In this study the essential oils from both cinnamon and ginger at concentration of 0.025%, 0.15% respectively suppressed the mycelia growth of *C. gloeosporioides* significantly as compared to the control. The chemical compositions of ginger essential oils are zingiberene was the

major compound, followed by geranial, β-bisabolene and ar-curcumene. With these it may contain around another 60-70 components in it. These are the responsible for the anti-microbial action of the ginger essential oil. With these it may contain around another 60-70 components in it. These are the responsible for the anti-microbial action of the ginger essential oil. The chemical composition of the cinnamon leaf oil has twenty five components representing 99% of the total oil.

Cinnamaldehyde was the major component which alone constituted around 90% of the oil. Other components like linalool, Z-cinnamaldehyde and α-pinene are the composed of around 1 %. These are the responsible for the anti-microbial nature of the cinnamon leaf essential oils antimicrobial activity. It was reported that both cinnamon oil and ginger oil added at 2% in potato dextrose agar (PDA) completely inhibited the growth of seven mycotoxigenic molds (*Aspergillus flavus, A. parasiticus, A. ochraceus, Penicillium sp, P. roqueforti, P. patulum*, and *P. citrinum*) for various incubation periods up to 21 days (Hortense et al., (2009)) and also inhibit the growth of yeasts(Conner DF and Beuchat CR (1984)). Suksrikarm B (1987) similarly reported that cinnamon oil and ginger oil separately inhibit many other microbes including *Lactobacillus* sp., *Bacillus thermoacidurans, Salmonnella* sp., *Corynebacterium michiganense, Pseudomonas striafaciens, Clostridium botulinum, Alternaria* sp., *Aspergillus* sp., *Canninghamella* sp., *Fusarium* sp., *Mucor* sp., and *Penicillium* sp . Hortense *et al.,* (2009) reported that, cinnamon was the most effective extract as it significantly inhibited the mycelial growth of *A. niger, F. sambucinum, P. sulcatum and R. stolonifer* by 63 to 100%. Aqueous cinnamon bark extracts had previously shown in vitro effects against a host of microorganisms, including *Aspergillus candidus, A. niger* and *Fusarium culmorum* found that ≤500 ppm of cinnamon oil can inhibit *Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium moniliforme* on PDA and Nguefack *et al*., (2004)reported the effect that cinnamon oil at concentrations of 1000 ppm completely inhibited the growth of *A. flavus.* Various aqueous and alcohol extracts or essential oil distillates from cinnamon leaves have also shown antimicrobial activity against *R. stolonifer* (Bosquez-Molinaa, E., et al., 2010) and *Fusarium oxysporum*, (Fawzi, E.M., et. al, 2009). This is due to their constituent of some compounds like Cinnamaldehyde 0.5% - 5%, Eugenol >75%, Eugenol acetate 1% - 4%, β-caryophylene 2%-5% (Pichersky et al., 2006).

4. Conclusions

In recent years, existing synthetic fungicides such as prochloraz and imazalil have become increasingly unpopular in post-harvest management. Consumers perceive these fungicides to be associated with risks, such as carcinogenicity, allergies, toxicity and environmental contaminations. For these reasons organic products have become more sought after in spite of the higher cost involved. In addition, many commercially important post-harvest pathogens have genetically adapted to synthetic fungicides, thereby reducing the efficacy of such preventative treatments. Many essential oils and their constituents have antimicrobial activities, rendering these natural products good alternatives to synthetic fungicides. Essential oils are considered as GRAS (generally regarded as safe) and would therefore be more acceptable to consumers. The multi component nature of essential oils makes it more difficult for pathogens to build up resistance. The application of essential oils as alternatives, or in addition to synthetic fungicides, can contribute to prolonging the useful life of these synthetic agents in the post-harvest environment. Since the C. *gloeosporioides* is the virulent causative agent of anthracnose of mango fruit, essential oils protect the growth of anthracnose or its development on mango fruits.

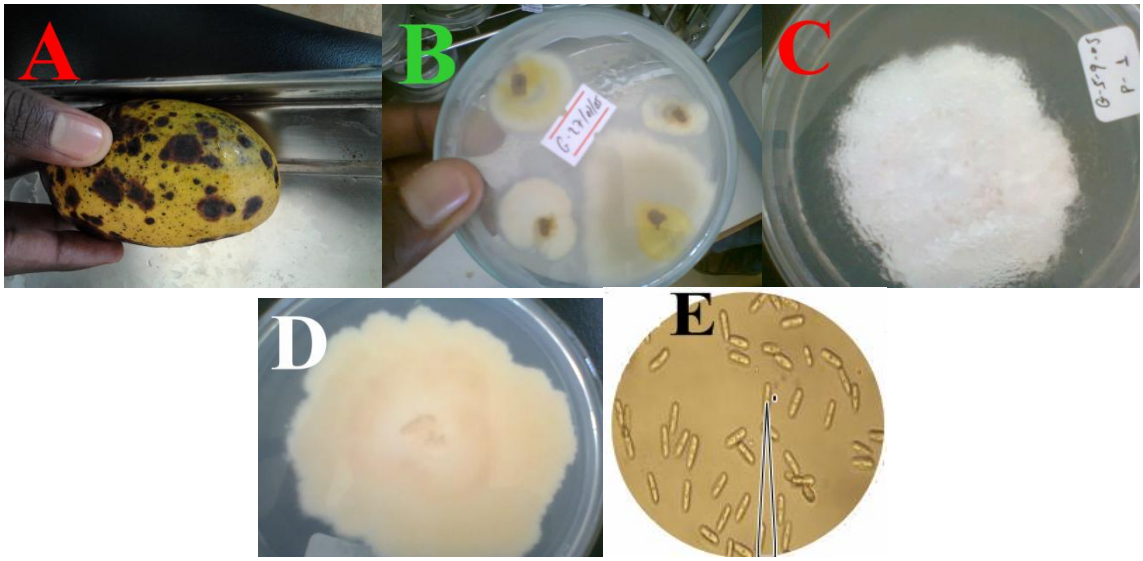

Figure 1. **(A)** *Colletotrichum gloeosporioides* symptom on mango fruit; **(B)** Colony structure on PDA; **(C)** Front side of *Colletotrichum Gloeosporioides*; **(D)** Back side of *Colletotrichum gloeosporioides* on plate after purification; **(E)** Morphological characteristics under microscope of 40 X lenses

Figure 2. Symptom of *Colletotrichum gloeosporioides* observed on mango fruit after six day of inoculating for Pathogencity test

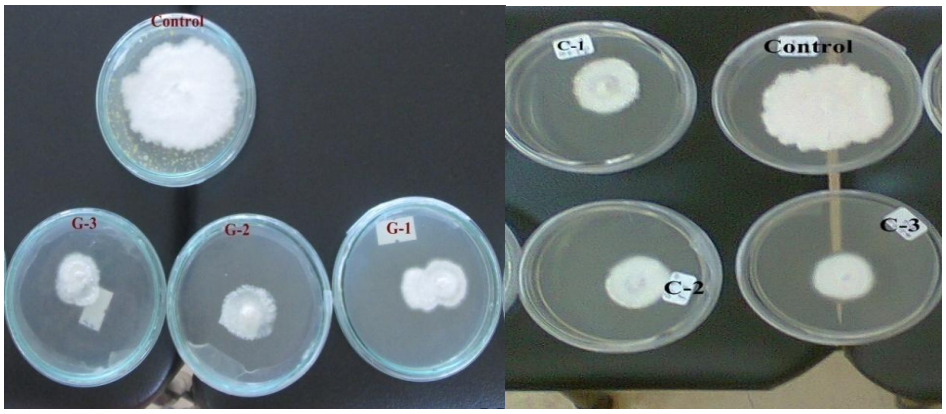

Figure 3. Effect of different concentration of ginger and cinnamon essential oils treatment on growth of *C. gloeosporioides as* compared to untreated control

Table 1. Effect of different concentrations of cinnamon and ginger essential oils on radial growth (cm) of *Colletotrichum gloeosporioides*

Sample	Essential oils concentration	Radial growth(Cm)	Inhibition percentage
1	C1	0.95^{b}	86.60
2	C2	0.77^{c}	89.13
3	C3	0.71^{dc}	89.98
4	G1	0.72^{c}	89.84
5	G2	0.66^{dc}	90.69
6	G3	0.61^{d}	91.39
7	CT	7.09^{a}	0.00
	LSD	0.15	
	CV	0.99	

Values followed by the same letters within the column are not significantly different at (α = 0.05)
C1=0.15%; C2=0.30%; C3=0.45% G1=0.0025%; G2=0.050%; G3=0.075%; CT= Control
LSD=least significant different, CV= coefficient variation

The minimum inhibition concentration of cinnamon and ginger essential oils for the development of mango anthracnose was below 0.025% and 0.15% respectively. Thus, the media mixed with essential oil at or above this concentration has clear inhibition effect over the control both *in vitro.*

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EXTRACTION AND EVALUATION OF ANTIOXIDANT POTENTIAL IN RAMBUTAN RIND AS FOOD WASTE

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ABSTRACT

The fruit of *Nephelium lappaceum* L., rambutan is a tropical fruit native to Southeast Asia. The rind of rambutan is normally discarded as food waste or by-product of food industry contains significant antioxidant properties. The aims of this study were to evaluate antioxidant potential of rambutan rind based on different extraction parameters. The oven-dried rambutan rind was extracted using different extraction parameters, such as solvent concentration (0-100%), extraction time (1-5 h), and extraction temperature (25-60°C). Based on the optimal levels of total phenolic content and DPPH radical scavenging activity obtained, the best extraction parameters (60% ethanol, 1 h extraction time, and 30°C extraction temperature) were selected to further determine for total phenolic content, and antioxidant activities in the rambutan rind. The rambutan rind extract had high total phenolic content (11.06 mg GAE/g sample) and moderately high level of DPPH scavenging activity with EC_{50} value of 49.12 µg/ml. It also had high β-carotene bleaching inhibition activity (93%), and moderate levels of lipid peroxidation inhibition activity using ferric thiocyanate (24.47%) and thiobarbituric acid (32.99%) methods. Due to the high total phenolics in rambutan rind extract and moderate to high levels of antioxidant activities, the rambutan rind could be a potent source of nutraceutical ingredient.

1. Introduction

Antioxidants are chemical compounds that can prevent or inhibit oxidation process. Antioxidants are including phenolic, carotenoid, xanthone, vitamin, and mineral. Phenolic compound is one of the phytochemicals with high antioxidant activity. Phenolic acid, flavonoid, and anthocyanin are the members of phenolic group. Phenolic compounds are predominantly found in most fruit species. In nature, a fruit is divided into endocarp, mesocarp and exocarp. Pericarp of most fruit are edible, while some of the non-edible exocarp (peel or rind) of the fruit is considered as waste. High phenolic compounds are commonly found in most fruits, especially citrus fruits. However, high total phenolic contents and antioxidant activities have been determined in citrus peels (Ghasemi et al., 2009). Among different parts of fruits studied using FRAP assay, the peels of pomegranate and hawthorn have relatively high antioxidant activities (Guo et al., 2003). High phenolics content was also determined in the rind of

rambutan (Khonkarn et al., 2010). Native to Southeast Asia, rambutan (*Nephelium lappaceum* L.) belongs to the same family (Sapindaceae) with lychee and longan. Rambutan is one of the attractive tropical fruit in Southeast Asia. The popular varieties of rambutan in Thailand are Rongrien and Seechompoo (Dembitsky et al., 2011). Thailand as the largest producer and exporter of canned rambutan utilized 2.48% of the total rambutan production (519,000 tonnes) for food industry in year 2005 (Chomchalow et al., 2013). Annually, more than 200,000 tonnes of rambutan rind are estimated to be discarded as by-product of food industry. Rambutan pulp contains gallic acid as one of the phenolic compounds, but it possesses low antioxidant activity (Gorinstein et al., 1999). Conversely, the discarded rinds of rambutan fruit have high phenolic antioxidants (Thitilertdecha et al., 2008). Rambutan rind is also considered as a potential source of natural antioxidants (Palanisamy et al., 2008). Previous literature reported that rambutan rind has high antioxidant and antibacterial activities (Zhao et al., 2011). Besides, Mokbe and Hashinaga (2005) reported that water extract of banana peel has antioxidant activity comparable to synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene. Therefore, it would be worthwhile investigating the nature of phenolic compounds present in fruit peel.

Electron-transfer and hydrogen atom transfer reaction assays are the commonly used methods in measuring antioxidant activity of plant extracts (Huang et al., 2005). DPPH assay is one of the typically antioxidant methods that involved electron-transfer reaction, while β-carotene bleaching and lipid peroxidation assays are involving hydrogen atom transfer reaction. Although Folin-Ciocalteu reagent method that commonly used to estimate total phenolic content in plant extracts is involved electron-transfer pathway, it is not a method for measuring antioxidant activity. Due to high total phenolics and antioxidant activity discovered in other variety of rambutan rind, therefore, this study aimed to evaluate potential antioxidant properties of rambutan rinds obtained as waste of rambutan fruit consumption in Malaysia.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals and reagents were analytical grade. Gallic acid, linoleic acid, Tween 40 (polyoxyethylenesorbitan monopalmitate), sodium carbonate anhydrous, chloroform and iron (III) chloride anhydrous were purchased from Fisher Scientific (UK). Folin-Ciocalteu reagent was from Merck (Germany). DPPH (2,2-diphenyl-1-picryhydrazyl), β-carotene, BHA (butylated hydroxy anisole) and α-tocopherol were purchased from Sigma-Aldrich (St. Louis, MO). Water used was of Millipore quality.

2.2. Preparation and extraction of rambutan rind

The rambutan fruit was purchased from a local supplier in Kuala Lumpur. Voucher specimens of *Nephelium lappaceum* (rambutan) had been deposited in the Herbarium of the National University of Malaysia. Only freshly harvested red rambutan fruits in good condition were obtained. The whole fruit was cleaned under running tap water, and the rind was separated from its pulp. The rambutan rind was collected as food waste after consumption of rambutan pulp. The rind was cut into small pieces of approximately 1 cm^2 and dried in a hot air oven (GmbH, Schwabach, Germany) at 45°C for 24 h. All the oven-dried rinds were ground into fine powder using a grinder (Sharp^{TM}, Malaysia) and stored at 20°C before further analysis.

Extraction of rambutan rind was carried out using a binary solvent extraction system as described by Chew et al. (2012). Briefly, the dried rambutan rind powder (5 g) was added with 50 ml of extraction solvent. Three extraction parameters (ethanol concentration, extraction time, and extraction temperature) were applied for extraction of antioxidants in

the rambutan rind. Based on simple optimization of extraction condition, single factor design was applied to determine the best extraction parameters for extraction of the rambutan rind.

The rambutan rind was first extracted using different percentages of ethanol (0-100%), and the extraction was performed at room temperature Tachakittirungrod et al., 2007) for 1 h. The selected percentage of ethanol was used to extract the rambutan rind at room temperature based on different extraction time (1-5 h). The analysis was further performed to determine the best extraction temperature (25-60°C) by applying the selected percentage of ethanol and extraction time. The best extraction parameters were selected based on the optimal levels of total phenolics and DPPH scavenging activity obtained from the rambutan rind extract. Extraction was performed in triplicate for each extraction condition.

2.3. Folin-Ciocalteu reagent assay

Ethanolic extracts of rambutan rind were determined for total phenolic content (TPC) using Folin-Ciocalteu reagents (Singleton et al., 1999) with slight modification. Before addition with 7.5% sodium carbonate solution, Folin-Ciocalteu reagent was diluted tenfold with deionized water. TPC of the rambutan rind extract was expressed as mg gallic acid equivalent (GAE) per gram rambutan rind based on gallic acid standard calibration curve (0.2-100 µg/ml). The calibration equation was:

$$y = 0.0165x\ 0.0003\ (R^2=0.9972) \quad (1)$$

2.4. DPPH radical scavenging activity

DPPH radical scavenging activity of the extracts was determined as described by Xu and Chang (2007) with some modifications. For preparation of DPPH radical solution, 7.8 g of DPPH powder was dissolved with 100 ml of ethanol. Different concentrations of the extract (5-30 µg/ml of sample) were prepared in different test tubes, followed by addition of 1 ml of DPPH solution and vortexed for few seconds. The reacting mixtures were keep in dark at room temperature for 30 min. BHA and α-tocopherol (200 µg/ml) were used for comparison, while the reagent mixture without rambutan rind extract was negative control. Applying the best extraction parameters, DPPH scavenging activity of the rambutan rind (1000 µg/ml) was determined and compared with BHA and α-tocopherol (200 µg/ml). Absorbances of the samples and control were measured at 517 nm. Radical scavenging activity of the rambutan rind extract was calculated using the equation as follows:

$$\text{Scavenging activity } (\%) = [1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}]x100 \quad (2)$$

2.5. β-Carotene bleaching assay

Inhibition activity of rambutan rind extract was measured using β-carotene bleaching assay (Yawadio et al., 2008) with slight modification. Before addition of the extract, 2 mg of β-carotene powder was dissolved in 2 ml of chloroform and added with 0.04 ml of linoleic acid and 0.4 ml of Tween 40 in a round bottom flask. The chloroform was removed under vacuum at 40°C for 10 min using a rotary evaporator (BUCHI, Switzerland). The mixture was added with 200 ml of deionized water, and then 1 ml of the mixture was added with 0.1 ml of the extract of different concentrations (10-200 µg/ml of sample). The mixture was incubated at 45°C and the absorbance (470 nm) was taken every 15 min for 120 min. Applying the best extraction parameters, DPPH scavenging activity of the rambutan rind (1000 µg/ml) was determined and the antioxidant standards (200 µg/ml) were used for comparison. The reagent mixture without the extract was used as negative control. Degradation rate (DR) was calculated according to first order kinetic using the equation as follows:

$$DR_{\text{sample}} = \frac{\ln(\frac{a}{b})x1}{t} \quad (3)$$

where: *a* is the initial absorbance at time 0, *b* is the absorbance at t = 20, 40, 60, 80, 100 or 120 min.

The inhibition activity (IA) was expressed as percentage of inhibition relative to the control based on the equation as follows:

$$IA(\%) = \frac{DR_{control} - DR_{sample}}{DR_{control}} \quad (4)$$

2.6. Lipid peroxidation assay

Inhibition of lipid peroxidation for the rambutan rind extracts were performed based on ferric thiocyanate and thiobarbituric acid assays.

2.6.1. Ferric thiocyanate (FTC)-based assay

FTC-based assay was performed based on a procedure described by Kikuzaki and Nakatani (1993) with slight modification. The rambutan rind extract (4 mg of sample) was first diluted with 4 ml of absolute ethanol, and added with 4.1 ml of 2.5% linoleic acid in absolute ethanol, 8.0 ml of 0.05 M phosphate buffer (pH 7.0), and 3.9 ml of deionized water. BHA and α-tocopherol (200 µg/ml) were used for comparison, while the reagent mixture without rambutan rind extract was used as negative control. The reacting mixture was incubated at 40ºC for 24 h before addition of ammonium thiocyanate. After 24 h of reaction, 0.1 ml of the mixture was added with 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. The mixture was further added with 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl after 3 min of incubation at room temperature. Absorbance of the red color solution was measured at 500 nm.

2.6.2. Thiobarbituric acid (TBA)-based assay

TBA-based assay performed based on the method described by Ottolenghi (1959) with slight modification. The rambutan rind extract (1 ml, 1 mg/ml) was added with 2 ml of 0.67% thiobarbituric acid and 2 ml of 20% trichloroacetic acid. BHA and α-tocopherol (200 µg/ml) were used for comparison, while the reagent mixture without rambutan rind extract was used as negative control. The mixture was placed in a boiling water bath (90ºC) for 10 min. After cooling, the mixture was centrifuged at 3000 rpm for 20 min and absorbance of the supernatant was read at 552 nm.

$$IA(\%) = [1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}]x100 \quad (5)$$

2.7. Statistical analysis

Data were expressed as means ± standard deviations and analyzed using SPSS version 19. One-way analysis of variance (ANOVA) and Tukey's multiple comparisons were used to determine the significant differences among the means, with significant level of $p<0.05$.

3. Results and discussions

3.1. TPC and scavenging activity based on different extract parameters

Evaluation of antioxidant potential in rambutan rind was first determined based on different extraction parameters. Extracted using different ethanol concentrations, extraction times, and extraction temperatures, TPCs of the rambutan rind extracts are presented in Figures 1-3. Assessed using DPPH assay, EC_{50} values of the rambutan rind extracted based on the extraction parameters are shown in Figures 4-6. As shown in Figure 1, TPC of the extract increased steadily until it reached a maximum recovery of TPC using 0% to 60% ethanol. Using higher ethanol concentration, no significant increment was found for the recovery of TPC. As the extraction time increase from 1 h to 3 h (Figure 2), the recovery of TPC significantly decreased but steadily increased at 4-5 h of extractions. For different extraction temperature applied (Figure 3), when extraction temperature increased to higher than 30°C, the recoveries of TPC did not show any improvement. As the recoveries of TPC were not significantly differed among 30, 50, and 60°C of heat treatment, DPPH scavenging activity was essential for choosing the best extraction temperature for further analysis.

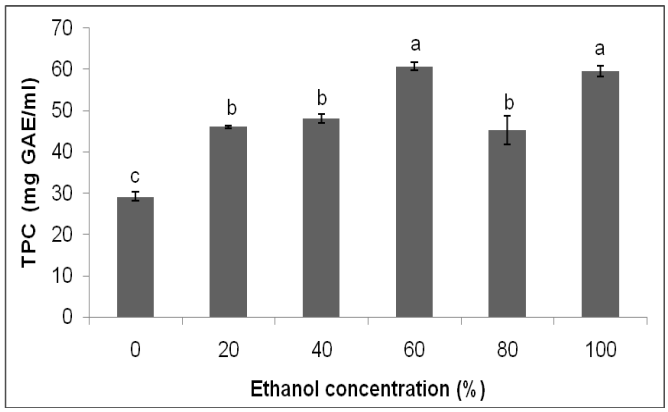

Figure 1. Effect of ethanol concentration on total phenolic compound (TPC) in rambutan rind. Values are presented as mean ± standard deviation of triplicate measurements. Different lower case letters (a-c) indicate significant differences at $p<0.05$.

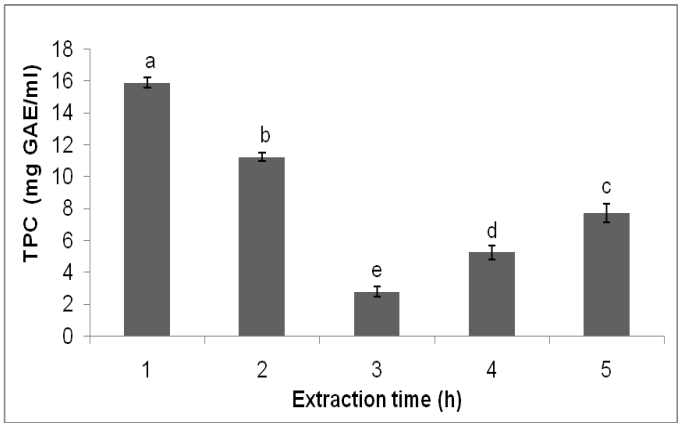

Figure 2. Effect of extraction time on total phenolic content (TPC) in rambutan rind. Values are presented as mean ± standard deviation of triplicate measurements. Different lower case letters (a-e) indicate significant differences at $p<0.05$.

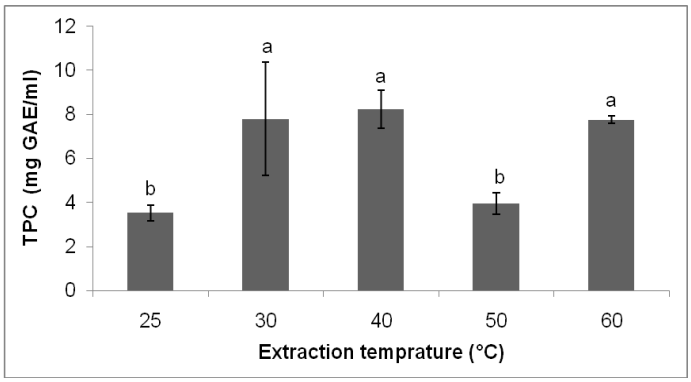

Figure 3. Effect of extraction temperature on total phenolic content (TPC) in rambutan rind. Values are presented as mean ± standard deviation of triplicate measurements. Different lower case letters (a-b) indicate significant differences at $p<0.05$.

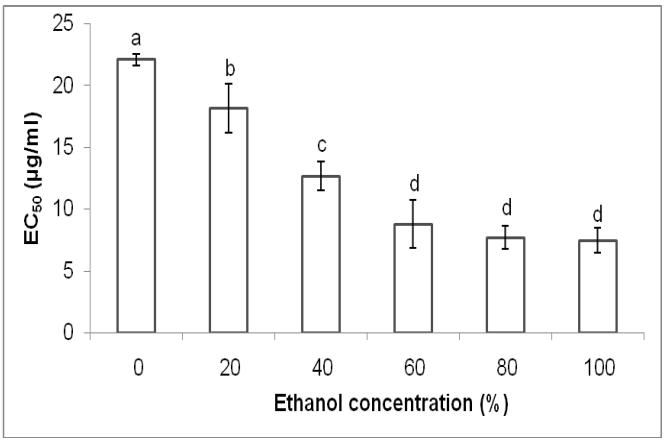

Figure 4. Effect of ethanol concentration on DPPH scavenging activity (EC_{50}) of rambutan rind. Values are presented as mean ± standard deviation of triplicate measurements. Different lower case letters (a-d) indicate significant differences at $p<0.05$.

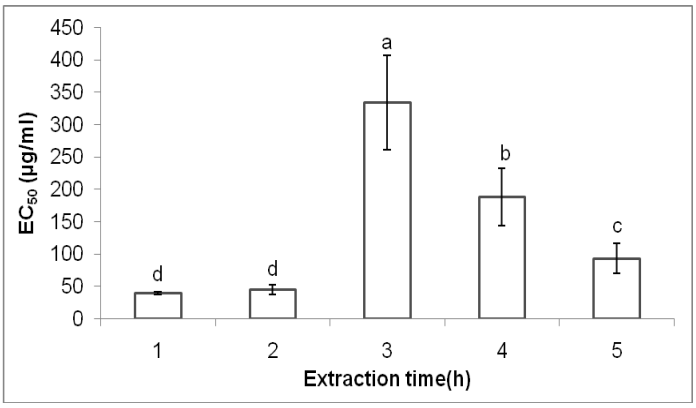

Figure 5. Effect of extraction time on DPPH scavenging activity (EC_{50}) of rambutan rind. Values are presented as mean ± standard deviation of triplicate measurements. Different lower case letters (a-d) indicate significant differences at $p<0.05$.

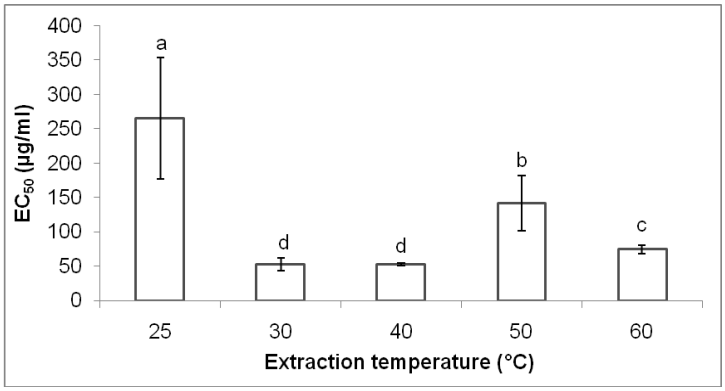

Figure 6. Effect of extraction temperature on DPPH scavenging activity (EC_{50}) of rambutan rind. Values are presented as mean ± standard deviation of triplicate measurements. Different lower case letters (a-d) indicate significant differences at $p<0.05$.

Table 1. Total phenolic content and antioxidant activities of rambutan rind

	DPPH (%)	Lipid peroxidation (%)		β-Carotene Bleaching (%)	TPC (mg GAE/g)
		FTC	TBA		
Rambutan	49.12±2.84[b]	24.47±8.51[a]	32.99±3.86[b]	93.12±6.85[a]	11.06±1.71
BHA	30.14±0.42[a]	22.99±9.07[a]	20.00±6.50[a]	90.11±3.11[a]	-
α-Tocopherol	55.24±0.68[c]	20.88±11.80[a]	18.38±12.62[a]	-	-

**Different lower case letters (a-c) indicate significant differences at p<0.05*

In this study, lower EC_{50} value obtained from DPPH assay indicated higher antioxidant activity. As shown in Figure 4, EC_{50} values of the rambutan rind extract had a decreasing trend when the ethanol concentration increased. However, no significant differences were found for the ethanol concentrations of 60, 80, and 100%. EC_{50} values of the extracts were the lowest if applying extraction times of 1 and 2 h (Figure 5). However, no significant difference was found for the EC_{50} values between 1 and 2 h. Similarly, extraction temperature of 30 and 40°C had the lowest EC_{50} values where the values were not significantly different (Figure 6).

Based on the results obtained, we can concluded that 60% ethanol, 1 h extraction time, and 30°C extraction temperature were considered as the best extraction parameters for optimal recovery of antioxidants in rambutan rind extract. Aqueous ethanol is commonly used for extraction of phenolic compounds that ranged from polar to semi-polar (Alothman et al., 2009). Turkmen et al. (2006) supported the fact that higher total phenolics were recovered from black tea extracted with lower percentage of ethanol compared to 80% and 100% ethanol. One hour extraction time is considered the best as Meyer et al. (1997) also supported that 1 h extraction of grape samples showed significantly higher antioxidant activity. Conversely, longer extraction time has caused oxidative degradation of phenolic compounds (Wu and Zhou, 2001). Although the 30°C extraction temperature showed higher standard deviation for the recovery of TPC from rambutan rind, extraction of plant sample at higher temperature has been found to increase polyphenoloxidase activity (Dincer, 2002). Besides, extraction temperature of higher than 30°C resulted in reduction of extraction yield (Cacace and Mazza, 2003). Moreover, at higher extraction temperature, the ability of antioxidants to react with free radicals also decreased (Reblova, 2012).

3.2. TPC and antioxidant activities based on the best extraction condition

After the first phase of simple optimization of extraction condition, the rambutan rind powder was further extracted using 60% ethanol, 1 h extraction time, and 30°C of extraction temperature. The TPC and antioxidant activity of rambutan rind extract (1 mg/ml of extract) was compared with antioxidant standards (BHA and α-tocopherol) (200 µg/ml).

The results showed that the rambutan rind extract had high TPC (11.06 mg GAE/g sample). Antioxidant activity of the rambutan rind extract determined based on DPPH assay was comparable to the antioxidant standards (Table 1). The rambutan rind extract had significantly higher DPPH scavenging activity (49.12%) compared to BHA (30.14%), but significantly lower than α-tocopherol (55.24%). The results also showed that inhibition activity (β-carotene bleaching assay) of the rambutan rind extract was comparable to BHA. Both of the extract and BHA has more than 90% of β-carotene bleaching inhibition activity. The lipid peroxidation inhibition activity of rambutan rind extract was also comparable with the antioxidant standards tested. As shown in Table

1, no significant differences were found for the FTC-based inhibition activity between rambutan rind extract BHA. Besides, the extract had significantly higher TBA-based inhibition activity compared to BHA. Okonogi et al. (2007) also reported that rambutan rind extract has higher antioxidant activity compared to mangosteen rind.

The FTC method indicates the amount of peroxide in the initial stages of lipid peroxidation, while the thiobarbituric acid method shows the amount of peroxide in the secondary stage of lipid peroxidation (Rahmat, et al., 2003). Thitilertdecha et al. (2008) reported that the antioxidant activity of rambutan peel extract was higher than its seed extract. Rezaeizadeh et al. (2011) also suggested the decrease in radical scavenging ability may be related to the increase in phenolic compound, where a linear correlation between total phenolic content and DPPH scavenging activity was found. Overestimation of TPC might be attributed by ascorbic acid that reacted with Folin-Ciocalteu reagent. The use of antioxidant assays that involved different reaction pathways has resulted variation in the antioxidant potential of rambutan rind extract. Different extraction conditions used for extracting potential antioxidants in the rambutan rind might also affect the antioxidant activity.

4. Conclusions

This study evaluated the TPC and antioxidant activity of rambutan rind that extracted based on the best extraction parameters. The extraction parameters of 60% ethanol, 1 h extraction time, and 30°C extraction temperature were selected due to the optimal TPC and low EC_{50} value of DPPH assay obtained for the rambutan rind extract. The rind of rambutan is a potential source of antioxidants due to the presence of phenolic compounds with TPC of 11.06 mg GAE per g rambutan rind. The antioxidant activities of rambutan rind extract were also comparable with the antioxidant standards. The rind of rambutan contained high total phenolics with high antioxidant activity allows it to be used as medicinal ingredient and source of nutraceutical. The rambutan rind extract, either alone or in combination with other bioactives, which also can be used in cosmetic and pharmaceutical applications.

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EVALUATION ON THERMAL, RHEOLOGICAL AND STRUCTURAL PROPERTIES ON THE MIXTURE OF POTATO STARCH AND PECTIN

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ABSTRACT

Starch is a common constituent of higher plants and it is the major form in which carbohydrates are stored. Starch has many applications in the food and non-food industries. Starch is constituted by two biopolymers: amylose and amylopectin. Each starch is unique. Starches also differ from each other. Several hydrocolloids has been used in the food industries as additives to improve stability and texture, for better water mobility and moisture control, to facilitate processing ant to improve the overall product quality and/or stability. Native starches do not have ideal properties for the preparation of food products and so "starch-hydrocolloids" combinations have been used in processed foods. In this investigation, commercial grade of potato starch and pectin (2.5%) were analysed by TG/DTG, DSC, RVA, XRD and SEM techniques. Although lower alterations in pasting properties (RVA) were observed and the gelatinisation enthalpy (ΔH_{gel}) was mainly affected with higher water ratios.

1. Introduction

Starch is the most important storage reserve carbohydrate in plants and it is a raw material from different botanical origins. Starches have found different applications and they are used by the food, paper, chemical, pharmaceutical, textile industries, etc., since ancient times. Starch is one of the most common natural polysaccharide. It is composed of two types of molecules: amylose (AM) and amylopectin (AP). The first is predominantly a linear polymer with α-1$\rightarrow$4 linked glucose molecules, whereas the second is a highly branched polysaccharide consisting of α-1$\rightarrow$4 linked glucose molecules and α-1$\rightarrow$6 linkages at the branch point (Leivas et al., 2013; Beninca et al., 2008; Lacerda et al., 2008). Unprocessed or native starches are restricted for application in today's advanced technologies. So processing is necessary to engender a range of functionality in agreement with industrial needs (Beninca et al., 2013). The hydrocolloids or "gums", are non-starchy polysaccharides with different structures, such as differences in branching, flexibility, molecular weight ranges, ionic charge, all of which influence their behaviour and the rheological properties of their solutions. Pectin is a complex and non-nutritive polysaccharide extracted from citrus, apples and other fruits. It consists mainly of partial methyl-esters of polygalacturonic acid and their Na, K, Ca salts (Be Miller, 2011; Ridley, et al., 2001; Xiaohong and Be Miller, 2002). Several gums are widely used in food industries as additives to improve some properties as stability and texture of foods, for better water mobility and moisture control, to reduce costs, to facilitate processing and to improve the overall product quality and/or stability. They are largely used in the food

processing industry combined with different starches to modify their main rheological and pasting properties and several studies had been described in the literature (Ridley et al., 2001; Xiaohong and Be Miller, Alberton et al., 2014; Babic et al., 2006).

In this investigation, the untreated potato starch (commercial grade) was used in mixture with pectin gum (commercial grade) solution at 2.5%, with continuous stirring by 60 minutes. After, the slurry was centrifuged and the solid was dried at 45ºC by 12 hours and then analysed.

2. Materials and methods

The untreated potato starch was from commercial grade. It was used in this investigation with the aim of verify the activity action of pectin gum (2.5%) when it was added to potato starch.

The thermogravimetric and derivative thermogravimetric curves (TG/DTG) were obtained using the thermal analysis system TGA-50 (Shimadzu, Japan), where the samples were heated from 25ºC to 650ºC using open alumina crucibles with mass around 7.0mg of each sample under a synthetic air flow of 150mL min^{-1} at a heating rate of 10ºC min^{-1}. The instrument was preliminarily calibrated with weight standard and with standard calcium oxalate monohydrate. All percentages of mass loss were determined using TA-60 WS data analysis software. The derivative thermogravimetric curves (DTG) are the first derivatives of TG curves; they were calculated and used in the determination of the points of the main mass loss. The DSC curves were obtained using a thermal analysis system model DSC-Q200 (TA-Instruments, USA). The DSC curves were recorded with the aim of studying the gelatinisation process in the following conditions: an air flow of 50mL min^{-1}, heating rate of 10ºC min^{-1} and samples weighing about 3.5 mg. Mixtures in the 1:4, 1:5, 1:6 and 1:8 ratio (starch:water w/w) were prepared and maintained for 60 minutes in order to equilibrate the moisture content. Aluminum crucibles were sealed with lid and carried out in order to study the gelatinisation process. The instrument was previously calibrated using standard of Indium with 99.99% purity, *m.p.* = 156.6 ºC, ΔH = 28.56 J g^{-1}.

The pasting properties of the samples were determined using the viscometer model RVA-4 (Newport Sci., Australia). A suspension of 3g (8% moisture) of starch in 25g of accurately distilled water underwent a controlled heating and cooling cycle under constant shear, where it was held at 50ºC for two min, heated from 50 to 95ºC at 6ºC min^{-1}, and held at 95ºC for 5 min, cooled to 50ºC at 6ºC min^{-1} and held at 50ºC for 2 min. At the beginning of the heating the swelling of the starch granules occurs, which causes an increase in the viscosity suspension (time and temperature pasting) when the polymers with low molecular weight (amylose molecules) begin to be leached from the granules. The viscosity peak was observed when the granules were completely swollen and while maintaining the pasting temperature at 95°C under constant agitation. The granules began to break down and solubilisation of the polymers provided a reduction of viscosity (break). Analysis followed, with a cooling cycle that caused the reorganisation of some amylose and amylopectin polymers increasing the opacity and viscosity of the paste in a process called setback (Beninca et al., 2013; Aggarwal and Dollimore, 1998), which occurs due to the strong tendency to form hydrogen bonds between adjacent molecules.

The micro-images of each sample were observed with high resolution using a Scanning Electron Microscope (SEM), model VEGA 3 (Tescan, Czech Rep.). The technique allowed us to observe the surface of the studied starches and it was possible to calculate the average diameter (d_a) of the granules (Alberton et al., 2014; Andrade et al., 2014).

X-ray diffraction powder patterns (XRD) were obtained by using an X-ray diffractometer, model Ultima 4 (Rigaku, Japan), employing CuKα radiation (λ = 1.541 Å) and settings of 40 kV and 20 mA. The scattered radiation was detected in the angular

range of 5-50º (2θ), with a scanning speed of 8º min^{-1} and a step of 0.06º. The degree of relative cristallinity was quantitatively estimated using Equation 1 and following the method described in the literature (Beninca et al., 2013; Andrade et al., 2014; Oliveira et al., 2014).

$$Xc = \frac{Ap}{(Ap + Ab)} .100 \quad (1)$$

where: *Xc* is the relative crystallinity; *Ap* is the peak area and *Ab* is the basis area.

All the analysis was made in triplicate. Analysis of variance (ANOVA) and Tukey's test were used to compare sample means with 95% confidence level ($p<0.05$) using SASM-Agri 8.2 Software.

3. Results and discussions

In Figures 1(a) and 1(b) are depicted the TG/DTG curves of the untreated potato starch and the potato starch treated with pectin solution at 2.5%, respectively. The initial mass of each sample was around 7.0 mg. As the behaviour of other starches in previous work (Leivas, et al., 2013; Beninca et al., 2008; Lacerda et al., 2008; Beninca et al., 2013; Xiaohong and Be Miller, 2002), the thermal decomposition occurs in tree main stages: the first due to dehydration, followed of stability, and a second and third in consecutive reactions that were attributed to decomposition and oxidation of the organic matter (amylose and amylopectin). As described in literature, previous researchers (Lacerda et al., 2008; Aggarwal et al., 1998) have observed that thermal treatment of starches normally leads to its depolymerisation, when the applied temperature exceeds 300ºC under oxidative atmospheres. The starch undergoes a series of irreversible alterations; the structural alteration initially takes polymer in the formation of pyrodextrins and at higher temperatures, the degradation of macromolecules leads to the formation of levoglucosan, furfural and volatile products of lower molecular mass, and finally, carbon products remain. Two main losses were observed, and the results showed similar profiles to other starches from different botanical sources (Andrade et al., 2014). In this work, were found the values of the depolymerisation at 231 and 219ºC, respectively, due to the experimental conditions: oxidant air atmosphere (150 ml min^{-1}) and heating rate of 10ºC min^{-1}, in the TG/DTG instrument. Differential scanning calorimetry (DSC) is a technique in which the change of the difference in the heat flow rate to the sample and to the reference sample is analysed while they are submitted to temperature alteration. In the study of gelatinisation process, upon 2.0 mg each starch sample was added 8, 10, 12 or 16 μL of distilled water, respectively. In Figure 2 are depicted the DSC curves. The obtained values of gelatinisation enthalpy (ΔH_{gel}) as well as the onset, peak and conclusion temperature (T_o, T_p, T_c) are collected in Table 2. As it can be observed, the ratio starch:water causes an increase in the gelatinisation enthalpy and when compared the action of pectin gum on starch, the addition causes a decrease in the gelatinisation enthalpy. Little effect on T_o and T_p was found in this investigation. The same behaviour was observed by Tester et al. (2003) in studies with corn, waxy maize and wheat starch treated with pectin at 2%. The pasting properties of the studied samples were obtained with the viscometer RVA-4 (Newport Sci., Australia) and these properties are shown in Figure 3. It was observed a viscosity decrease in the pasting temperature and in the viscosity peak, when to the starch sample was added pectin gum. The pasting temperature followed the same behaviour of the "onset" temperature (T_o) observed in the DSC curves as verified by other authors (Beninca et al., 2008; Beninca et al., 2013; Lacerda et al., 2008). The RVA results are collected in Table 3. The X-ray diffractograms demonstrated that the untreated and modified starch with pectin showed lower changes in relative cristallinity, as can be seen in Figure 4 and the obtained results in Table 3. As expected, this behaviour is due to interaction of amylose and amylopectin with the pectin gum.

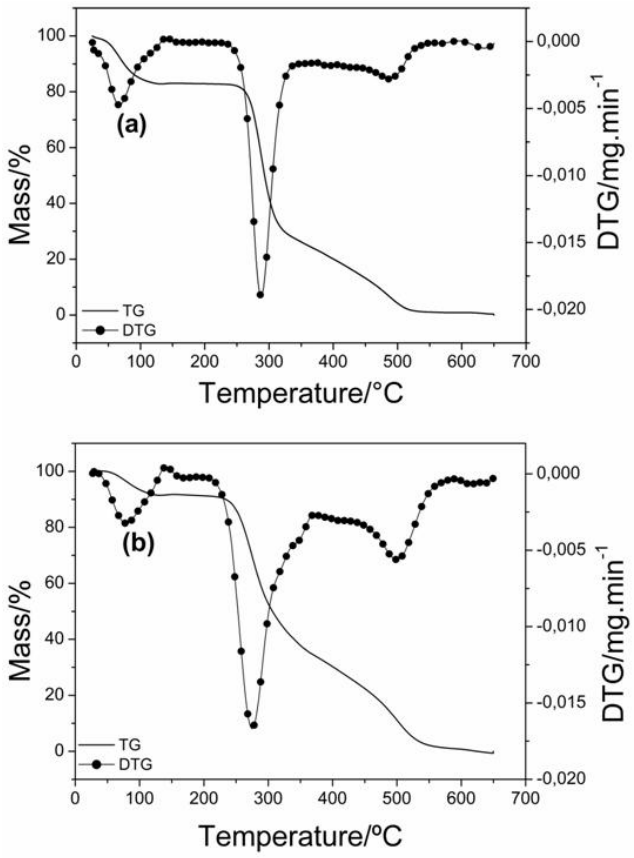

Figure 1. TG/DTG curves of (a) untreated potato starch and (b) potato starch treated with pectin 2.5%.

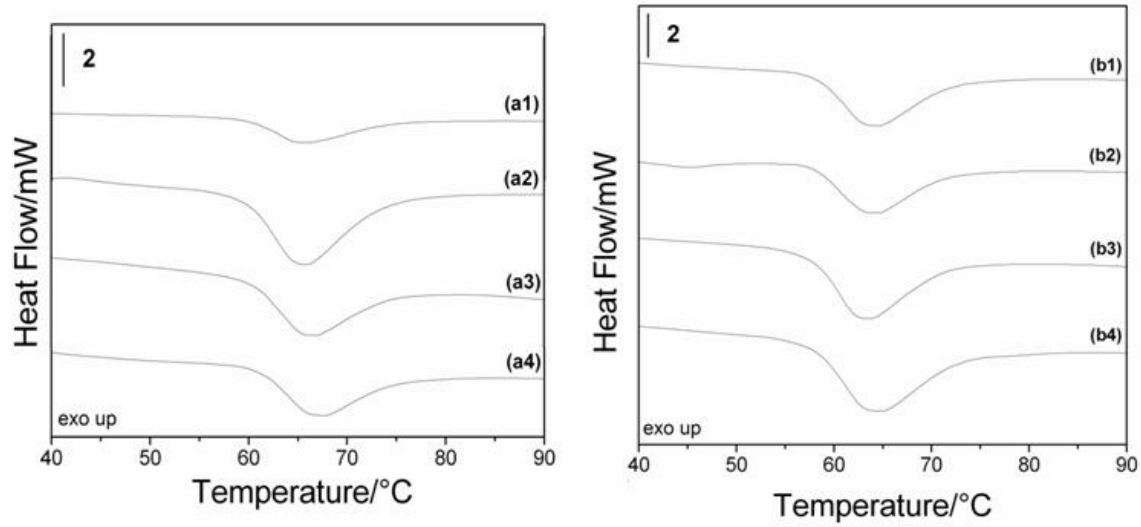

Figure 2. DSC curves of untreated potato starches with different proportions of starch:water (a1) 1:4, (a2) 1:5, (a3) 1:6, (a4) 1:8; and potato starch with pectin 2.5% with different proportions of starch:water (b1) 1:4, (b2) 1:5, (b3) 1:6, (b4) 1:8.

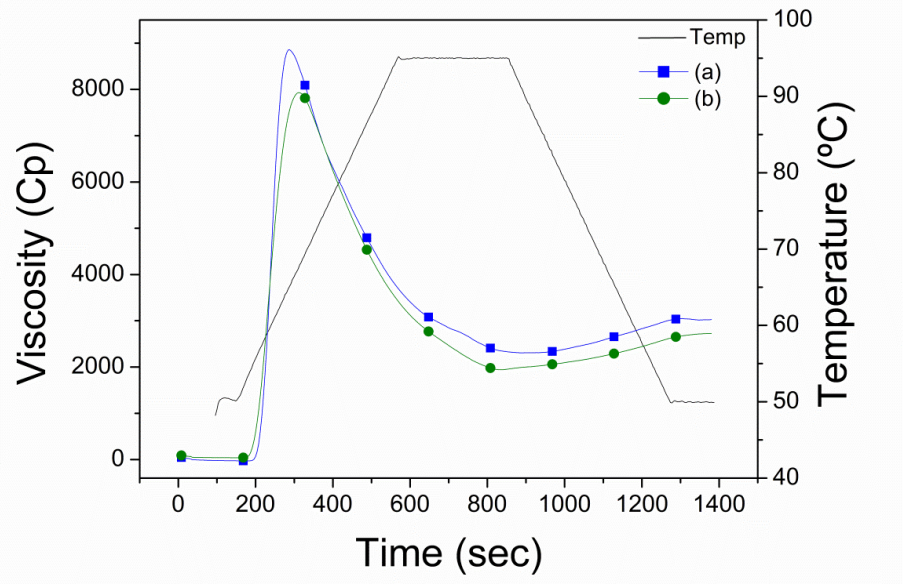

Figure 3. RVA results of: (a) untreated potato starch and (b) potato starch treated with pectin 2.5%.

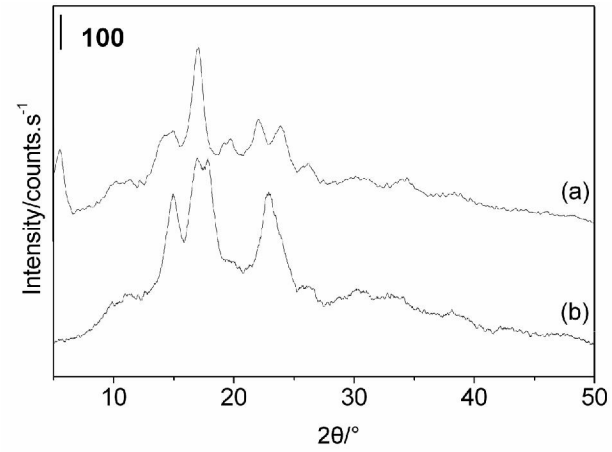

Figure 4. XRD diffractograms of: (a) untreated potato starch and (b) potato starch treated with pectin 2.5%.

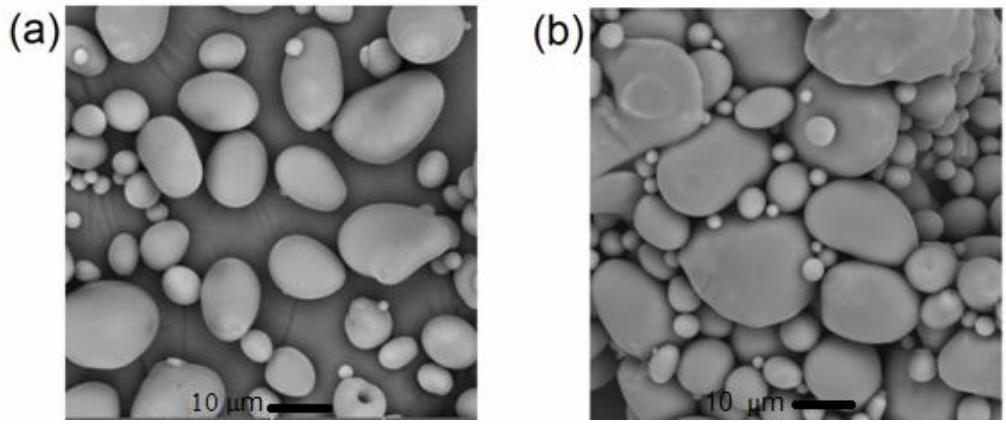

Figure 5. SEM microimages of: (a) untreated potato starch; (b) potato starch treated with pectin gum 2.5%. Magnification 750 X.

Table 1. TG and DTG results of: (a) untreated potato starch; (b) potato starch treated with pectin at 2.5%.

Sample	TG Results		DTG Results	
	Steps	***Δm*/%**	***ΔT*/ºC**	***Tp*/°C**
(a)	1st	16.92	30-170	67.00
	Stability	-	170-231	-
	2nd	58.81	231-368	287.60
	3rd	23.81	368-574	486.30
(b)	1st	8.59	30-182	79.49
	Stability	-	182-219	-
	2nd	57.65	219-373	274.89
	3rd	32.97	373-586	499.92

(*)Δm mass loss, (%), ΔT temperature range, T_p peak temperature.

Table 2. DSC results of untreated potato starches with different proportions of starch:water (a1) 1:4, (a2) 1:5, (a3) 1:6, (a4) 1:8; and potato starch with pectin 2.5% with different proportions of starch:water (b1) 1:4, (b2) 1:5, (b3) 1:6, (b4) 1:8.

Samples	*To*/ºC	*Tp*/ºC	*Tc*/ºC	ΔH_{gel}/J.g^{-1}
(a1)	60.27±0.02^{c}	65.46±0.03^{d}	73.95±0.08^{a}	14.02±0.11^{d}
(a2)	58.53±0.05^{d}	65.85±0.02^{c}	73.82±0.13^{a}	16.06±0.11^{c}
(a3)	60.37±0.02^{b}	66.64±0.03^{b}	73.46±0.05^{b}	12.89±0.05^{e}
(a4)	61.16±0.03^{a}	66.97±0.03^{a}	74.03±0.06^{a}	14.16±0.23^{d}
(b1)	58.39±0.04^{e}	63.97±0.03^{e}	71.48±0.12^{c}	10.17±0.33^{f}
(b2)	58.00±0.25^{g}	63.90±0.01^{f}	70.84±0.03^{d}	10.34±0.13^{f}
(b3)	57.95±0.01^{g}	63.21±0.02^{g}	70.93±0.06^{d}	20.64±0.37^{b}
(b4)	58.16±0.03^{f}	63.93±0.02^{ef}	65.70±0.03^{e}	22.81±0.11^{a}

(*) T_o "onset" initial temperature, *Tp* peak temperature, T_c "endset" conclusion temperature, ΔH_{gel} gelatinisation enthalpy. Averages followed by the same letters in the same column do not differ statistically by Tukey's test ($p<0.05$).

Table 3. RVA and XRD results of: (a) untreated potato starch; (b) potato starch treated with pectin 2.5%

RVA							XRD
Samples	**Pasting temperature/ °C**	**Viscosity peak/cP**	**Peak time/sec**	**Setback/cP**	**Break/cP**	**Final viscosity/cP**	**Degree of relative cristallinity**
(a)	64.88±0.81^{a}	9100.10±0.14^{a}	283.90±0.14^{b}	735.00±4.24^{b}	6799.00±4.24^{a}	3036.08±0.11^{a}	28.98±0.009^{a}
(b)	63.55±0.71^{a}	8050.05±0.07^{b}	311.85±0.21^{a}	798.75±0.35^{a}	6121.10±0.14^{b}	2728.03±0.04^{b}	25.81±0.005^{a}

cP "centipoises", sec "seconds". Averages followed by the same letters in the same column do not differ statistically by Tukey's test ($p<0.05$). The degree of crystallinity was calculated as a percentage, peaks are determined in 2θ average roughness.

The main diffraction peaks occurred at 2θ: (a) 11.1, 14.9, 17.0, 17.8, 19.7, 22.1, 23.9 and 26.2º; (b) 9.9, 11.4, 14.9, 16.9, 17.8 and 22.9º. The calculated degrees of relative cristallinity using the Equation 1 were: (a) 28.98% and (b) 25.81%, respectively.

The SEM microimages of the untreated and modified starch granules show the typical characteristics of potato starch with oval shape and no alterations were observed. Potato starch had granules between 5 and 110μm. The average diameter was calculated and were: (a) 37.32 and (b) 34.92 μm, respectively.

4. Conclusions

TG/DTG curves of the studied potato starches showed similar behaviour to other starches with mass loss in three main stages: dehydration followed of stability and decomposition and oxidation or amylose and amylopectin. The DSC technique let to obtain the values and temperatures of gelatinisation enthalpy that were higher with addition of pectin and water. The rheological profiles of the modified starch with pectin had lower values of viscosity peak, retrogradation and pasting temperature. The relative crystallinity and the starch granules morphology were not altered.

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QUALITY CHARACTERISTICS AND SENSORY EVALUATION OF APRICOTS JAMS MADE WITH DATE PALM PRODUCTS (SYRUP)

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ABSTRACT

The purpose of this research was to obtain a new formulation of jam apricot. The new method is the use of date syrup as replacement of sucrose. Common dates (Mech-Degla and Degla-Beida), containing a high sugar amount (66.21-69.32%) were used. Our study has highlighted the possibility of transforming dates in syrup, in order to use it as a substitute of sucrose. Syrups prepared from the two dates had a good nutritional value, they were characterized by high sugar content (66.24-66.28%), a total acidity (0.25-0.40%), ash values varied from (1.20-1.77%) and pectin (1.31-1.43%). Jam was prepared by mixing date syrups and apricot puree (1/1: w/w), it was heated under vacuum at 70°C to a final Brix of 62%. Gelling jams occur without added pectin and organic acids, which classify this product in natural food class without additives. Significant differences between the two jams studied ($p<0.05$) were noted for the water content, pH, total acidity, sugars, protein, ash, potassium, magnesium, calcium and copper. The organoleptic tests showed that this new formulation: apricot jam produced with Mech-Degla syrup, had a higher global acceptability (7.19) compared to apricot jam standard (6.91). Taken together, all results indicate the possibility of using date syrups as promising substituting sucrose in the formulation of jams and open broad prospects in food industries.

1. Introduction

The palm date (*Phoenix dactylifera* L.) is an important tree in the desert regions of North Africa and the Middle East countries. It plays an important role in the ecological and socio-economic plan. The fruits are an excellent food of great nutritional value and energy where sugars are the major constituents; they represent about 70 to 80% (Amira et al., 2011). In addition, dates contain other nutrients such as protein (2.3-5.6%), lipids (0.2-0.5%), ash (1-1.9%), fibers (6.4-11.5%), vitamins (vitamin C, B1, B2, ribovlavine A and niacin), organic acids and polyphenols (Booij et al., 1992; Al-Shahib and Al-Marshal, 2003; Barreveled, 1993; Al-Farsi et al., 2005; Vayalil et al., 2012). Furthermore, dates show therapeutic effects; they facilitate intestinal transit through their high dietary fiber and have an interesting antioxidant activity due to phenolic compounds, vitamins (C and E), carotenoids and selenium.

Algeria is the 6th largest producer of dates, with a rich genetic heritage attending 940 cultivars (Hannachi et al., 1998) and an annual production of about 552765 tons of dates (FAO, 2009), approximately 48.21% of the production consists of the famous variety:

Deglet-Nour (MARD, 2010), the rest is common dates (second grade), which are less appreciated by consumers and are not valued, they are generally devoted for animal feed. Recently, there has been an increase in the production of Deglet-Nour, to supply a growing demand for this cultivar in spite of the common varieties (Mech-Degla, Degla-Beida and Tantboucht). This has led to a weakening of biodiversity of palm cultivation systems. To face this threat, it is important to implement a processing industry for less commercial quality dates by relatively simple technological processes which will help to find serious opportunities for the date palm farmers to fully meet the requirements of socio-economic desert and reinvigorate these regions.

Several studies have shown the possibility of converting dates in various food and non-food products of high value and easily marketable as syrup, juice, vinegar, jams and alcohol (Espiard 2002; Al-Farsi et al., 2007; Benamara et al., 2008; Cheikh Rouhou et al., 2006). The date syrup is a high nutritional value food product, it can be consumed directly or used as ingredients in some food products formulations such as ice cream, beverage, confectionery and bakery (Barreveld, 1993; Razavi et al., 2007; Roukas and Kotzekidou, 1997).

This study aims to transform the common dates (Mech-Degla and Degla-Beida), available in large quantities and with interesting prices, into syrup, itself used as sucrose substitute in the formulation of apricot jam (mixture of apricot puree and date syrup). The physicochemical characteristics of the two produced jams are determined, and a sensory evaluation is carried out to judge the organoleptic quality with respect to a commercial apricot jam, and show the influence of the sum of syrups of dates on the acceptability degree of consumers of produced jams.

No study until now, to our knowledge, was devoted to the incorporation of syrups dates as replacing sucrose in the formulation of jams (a mixture of dates syrups and fruit), that makes the originality of our work.

2. Materials and methods

2.1. Vegetal material

The dates chosen for this study were dried dates of Mech-Degla and Degla-Beida varieties. They came from Algerian South-East region. They are harvested at full maturity and stored at 4 °C until analysis.

The variety of apricot retained in our study is widespread (variety of Ouardi), it is spherical in shape, more or less flattened on both poles and has a beautiful yellow and red color, very good taste, Their flesh is firm, juicy and flavorful. This variety comes from Aures region (Bouzina, Wilaya of Batna, Algeria).

2.2. Date syrup preparation

First, dates were sorted, cleaned and pitted. Obtained flesh was crushed and mixed with distilled water at a ratio of 1/3 water (W/V). The mixture was brought to a water bath at 72 °C for 30 min with periodic agitation. Obtained dates Extract was then filtered through a 50 microns cloth. Later, the filtered extract was concentrated under vacuum at 70 °C until the formation of 70 °Brix syrup.

2.3. Apricot preparation

Apricots (*Prunus armeniaca* L.) were cleaned and peeled, then exposed to steam for 5 minutes to inactivate enzymes that catalyze the browning or oxidation during grinding fruit and storage.

2.4. Formulation of the apricot jam with syrups dates

Apricot jam has been made by using date syrup instead of sucrose, the composition was as follows: 50% of date syrup and 50% apricot puree (weight/weight). No Additives were used. Jam baking is carried out at 70 °C under vacuum to 62 Brix. It was then pasteurized at 90 °C.

2. 5. Analytical methods

The moisture content was determined by drying an alicot in a vacuum oven at 70 ± 2°C, to constant weights. The pH was measured by a pH meter, type HANNA HI 2210, at 20 °C. The titrable acidity (expressed as g of citric acid per 100g of sample) is determined by the titration with NaOH 0.1N using phenolphtalein as an indicator according to AFNOR official standards (1984). Total sugars were determined by the phenolic sulfuric acid method (Dubois et al., 1956). Sugars were extracted (3g with 70 mL of distilled water) for 30 min at 70 °C (stirring frequently). After clarification of the aqueous extract, the total carbohydrate present was measured by colorimetry at 490 nm, using spectrophotometer type Shimadzu. A soluble solid was determined using an Abbe Refractometer, at 20 °C. The protein content was determined by Kjeldahl method (AFNOR), using a conversion factor of 6.25. The ash is determined by combustion of the sample in a muffle furnace (Heraeus), at 525 °C for 12 hours (AOAC, 1995). Minerals (Calcium, magnesium, potassium, sodium and copper) were measured by atomic absorption spectrometry on a Perkin Elmer 100 Analyst device, after nitro acid digestion (HNO_3:1M) and appropriate dilution. Pectins were determined by aluminum chloride complexation according to the method described by Joslyn and Luca (1956).

The syrups purity percentage was determined as follows: Purity = (Total sugar / Rate SS) x 100 (Mathlouthi et Reizer, 1995).

Polyphenols extraction and measurement

An amount of 1g of jam, was macerated in 40 mL of methanol with continuous agitation for 24 hours. The mixture was filtered then the filtrate was evaporated using a rotary evaporator Type: Heidolph. The total polyphenols were determined colorimetrically according to the method described by Meda et al. (2006): 500 µL of methanolic dates extract was added to 2.5 mL of Folin-Ciocalteu reagent (0.2N). The mixture was agitated for 5 minutes, and then neutralized with 2 mL of Na_2CO_3 (7.5%). absorbance at 760 nm is performed after incubation for 2 hours at room temperature in darkness. The phenolic content is expressed as mg equivalent of gallic acid (EGA) per 100g of fresh weight.

Reducing power

The reducing power was measured according to method described by Allane and Benamara (2010). A sample (1 mL) of each prepared jam as mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.60) and 2.50 mL of an aqueous solution of hexacyanoferrate potassium $K_3[Fe(CN)_6]$ at 1%. After 30 minutes of incubation at 50°C, 2.5 mL of trichloroacetic acid solution 10% was added and the mixture was then centrifuged at 10000 rev/min for 10 minutes (centrifuge SIGMA 2-16 PK). 2.5 ml of the supernatant was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ aqueous solution, the absorbance measured at 700 nm (UV/VIS). A higher absorbance indicates a high antioxidant activity. The results were expressed as mg equivalent of ascorbic acid per 100g of fresh date.

2.6. Sensory analysis

We conducted this test in order to assess the acceptability of apricot jams made using date syrup and the commercial apricot jam. Samples are coded and presented to degustation panel. Jams were evaluated for appearance, texture, color and taste, using a hedonic scale of 9 points. The scale interval fluctuates from ‘not pleasant’ to ‘very pleasant’ (Le Magnen, 1998). Evaluation was made by a group of trained and untrained panel (students of department of food technology, University of Batna) aged between 20 to 35 years old.

2.7. Statistical Analysis

Parameter values are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS version 20 software. The Duncan's test is used to evaluate the significance of differences between mean values at p□ 0.05.

3. Results and discussions

3.1. Physicochemical fruits composition

Table 1 shows the physicochemical composition of date varieties. Dates are characterized by their high sugar content; ranging between 69.32 and 66.21% of the fresh weight. These results exceed limit indicated by Ben Ismail et al. (2012), who reported values between 44 and 62.70 % for Tunisian varieties, showing that they are of high energy value.

The content of reducing sugars recorded for the date of Mech-Degla is 14.29 %, it is lower than the date of Degla-Beida, which is 46.27% of fresh weight, with a significant difference. According to Al-Farsi and Lee (2008), 100g date pulp can provide 213-314 Kcal. The titrable acidity of the two studied dates varieties were respectively 0.36 and 0.54% for Mech-Degla and Degla-Beida. These results are much higher than those cited by Khalil et al. (2002), giving a value of 0.18 and 0.22 % (citric acid equivalent), respectively, for the Siwi and Amhat Egyptian varieties. The ash rate was between 2.13 and 2.47% of the dry weight, similar to those reported by Sawaya et al. (1983), with respectively 2, 2.2 and 2.6 for Sifri, Barni and Ruzeiz Saudian varieties. Duncan test revealed that the two date variety were significantly different in total acidity and ash content. The difference in the biochemical composition recorded between date varieties depends on several factors such as: variety, soil type, fertilization, irrigation and climate conditions (Yousif et al., 1982; Booij et al., 1992, Ismail et al., 2006; Amira et al., 2011).

Table 2 gives the physicochemical composition of used apricot. The registered dry matter of the used apricot variety was 15.78 %. This value is comparable to those found by Madrau et al. (2009), which gave values of 15.71 and 16.09% respectively for the two varieties grown in Pelese Cafona and Italy. The soluble solids (°Brix), was about 15.58%, this result is consistent with those given by Ali et al. (2011), who reported a range of 12.67 to 20 °Brix for some Pakistanian varieties.

The pH was 03.63, which is consistent with those reported in the literature (Lo Voi, 1995). The ratio TSS/acidity was equal to 6.46, it is within the limits given by Hegedus et al. (2010). Sugars occurred with a rate of 10.24% of the fresh weight; this value is higher than that quoted by Ragab (1987), which gives 7.15%. The rate of ash was 4.32% of the dry weight; it is comparable to Haciseferogullari et al. (2007) data, placed within the limits of 2.72-5.34%. The pectin content of our apricot variety is 1.86%. This amount is higher than results reported by Baker (1997), which was 0.71-1.32%. Indeed, the apricot is a fruit rich in pectin, fact revealed by several studies.

3.2. Physicochemical composition of date syrups

The physicochemical composition of prepared date syrups is given in Table 3.The biochemical composition of date syrups shows that sugars were the major components, with a rate between 66.28 and 66.24%, but without a significant difference between the two syrups ($p \square 0.05$). The reducing sugar content was recorded as 22.39 and 65.75% for the date syrups of Mech-Degla and Degla-Beida respectively, with a significant difference. These differences appear to be due to varieties of dates (table 1).Whoever, acidity and thermal treatment applied during the concentration accelerated the conversion of sucrose into reducing sugar (Ben Thabet et al., 2009; Besbes et al., 2011).

The higher ash content was recorded for Degla-Beida syrup, the two groups were significantly different, but the date syrups ash content is higher than maple syrup and honeys. The mineral profile of date syrups was characterized by the abundance of potassium, fact that has been reported in several studies (Khalil et al., 2002; Abbes et al., 2011). Significant differences were observed in potassium, magnesium, calcium and copper levels ($p< 0.05$). By comparison to white sugar, date syrups are richer in mineral components, sugar containg just traces: 2.2, 0.6 and 0.2 mg/100g respectively for potassium, calcium

and magnesium (Vierling, 2003). The date syrups pectin rate was within the range of 1.31-1.43 % of the fresh weight, with a non significant difference between groups. These results are similar to that given by Alanazi et al. (2010), which give 1.46% for 'Khalas' date syrup (Saudi Arabia).

The whole results show that the date syrup is a good source of nutrients: rich in sugars, minerals and pectin. It is a promising product that offers several advantages compared to sucrose syrup, and it can be therefore incorporated into food and pharmaceutical preparations (Khalil et al., 2001; Alanazi, 2010).

3.3.Physicochemical characteristics of apricot jams

The average composition of jams prepared with the two date syrups is mentioned in table 4. Jams contain water content below 40%, which is favorable for good preservation (Espiard, 2002; Fredot, 2005). Apricot jam (Degla-Beida syrup) contains a dry matter content lower than jam made of Mech-Degla syrup, with a significant difference between the two preparations. The sugar rate of jams was found between 50.50 and 54.52%, with the lowest value recorded for Degla-Beida date preparation. These sugar levels are comparable to those reported in the literature (Ciqual, 2012). Obtained jams pH was between 3.86 and 4.02, with a significant difference. These pH values causes sucrose partial inversion and limit sugar crystallization (Besbes et al., 2010). The pH should be between 3 and 3.5. A lower value would have disadvantages: too far inversion causing glucose crystallization or too fast gelling with lumps, with excessive acidity and exudation (Cheftel and Cheftel, 1976). According to tables 3, 4 and 5, dates, jams and syrups total acidity was inversely proportional to the pH values. The jam total acidity is greater than the dates and syrups acidity because of the participation of acids from all ingredients (table 1 and 3).

Protein was about 0.46-0.85%. Degla-Beida syrup Jam was richer in protein. These values are slightly higher than those reported in the table of food composition (Ciqual, 2012), reported concentration of 0.3-0.70%. Prepared jams contain ash rates ranging between 1.37-1.66% of the dry weight, with a significant difference between the two preparations ($p \square 0.05$). The highest level was noted in Degla-Beida syrup jam, this is due to the richness of Degla-Beida date varietie in mineral components (table 1). Jams mineral profile brought out that potassium was the major constituent (620.23-835.08mg/100g) with a significant difference, with the highest value recorded for the jam elaborated by Degla-Beida syrup. A significant difference was also observed for the copper, calcium and magnesium, with highest values observed for the Degla-Beida syrup jam. The mineral contents of date syrups jams were much higher than those recorded for the apricot jam made from sucrose, which are 114, 9.1, 5 and 0.1mg/100 g for potassium, calcium, magnesium and copper respectively (Ciqual, 2012).

Prepared jams were made without added commercial pectin and citric acid. The jellification was in our case due to pectin and organic acids that occur naturally with sufficient quantities in apricot and date syrups. This allows us to classify jam using date syrup as a natural product without additives and with high added value.

The results of the polyphenls and reducing power of jams apricot are mentioned in Table 5. Polyphenols are natural biologically active antioxidants (Kwang Ang et al., 2012), they warn against degenerative and neurodegenerative diseases such as cancer, atherosclerosis and Parkinson's (Ndhlala et al., 2007; Ben Thabet et al., 2009). Mech-Degla syrup Jam contains a little high phenolics content (122.20 mg/100g), followed by jam of Degla-Beida syrup (113.53 mg/100g), corresponding respectively to reducing power of 201.60, and 185.32 mg of ascorbic acid equivalent. Elaborated jams using date syrups are a good source of polyphenols and can be considered as a functional food.

Table 1. Physicochemical characteristics of date varieties

Parameters	Mech-Degla	Degla-Beida
Dry matter (%)[1]	88.01± 0.11 a	88.36 ±0.037 a
Total sugars (%)[1]	69.32 ± 1.15 a	66.21 ± 2.49 a
Reducing sugars (%)[1]	14.29 ± 0.39 a	46.27 ± 0.40 b
Titrable acidity (%)[1]	0.36 ± 0.04 a	0.54 ± 0.01 b
Ash (%) [2]	02.13± 0.07 a	2.47 ± 0.005 b

The different superscript letters within the same line are significantly different at $p < 0.05$.
[1:] Expressed of the fresh weight.
[2:] Expressed of the dry weight.

Table 2. Physicochemical characteristics of apricot

Parameters	Values
Dry matter (%)[1]	15.78 ± 0.02
pH	03.63 ± 0.01
Titrable acidity (as citriq acid,%) [1]	02.41 ± 0.05
Total soluble solids (°Brix)	15.58 ± 0.14
Total sugar (%)[1]	10.24 ± 0.14
Reducing sugars (%)[1]	2.62 ± 0.15
Ash (%) [2]	04.32 ± 0.03
Pectin (%)[1]	01.86 ± 0.21

[1:] Expressed of the fresh weight.
[2:] Expressed of the dry weight.

Table 3. Physicochemical composition of date syrups

Parameters	Mech-Degla Syrup	Degla-Beida Syrup
Water (%)[1]	23.83 ± 01.15 a	27.66 ± 0.28 b
Dry matter (%)[1]	76.16 ± 01.15 a	72.33 ± 0.28 b
pH	05. 19 ± 0.00 a	04.61 ± 0.01 b
Titrable acidity (as citric acid,%) [1]	0.25 ± 0.00 a	0.40 ± 0.01 b
Total soluble Solids (°Brix)[1]	70.00 ± 0.00 a	70.00 ± 00.00 a
Total sugars (%)[1]	66.24 ± 01.94 a	66.28 ± 01.90 a
Reducing sugars (%)[1]	22.39 ± 2.20 a	65.75 ± 3.16 b
Purity %	93.95 ± 2.75 a	94.01 ± 2.70 a
Ash(%) [2]	1.20 ± 0.08 a	1.77 ± 0.03 b
Potassium (mg/100g) [2]	513.69 ± 01.98 a	693.43 ± 20.36 b
Magnesium (mg/100g) [2]	43.59 ± 2.13 a	50.08 ± 0.01 b
Calcium (mg/100g) [2]	21.06 ± 0.05 a	19.01 ± 0.05 b
Copper (mg/100g) [2]	0.55± 0.00 a	0.74 ± 0.05 b
Pectin (as pectate d'Al,%) [1]	01.43± 0.19 a	1.31 ± 0.24 a

The different superscript letters within the same line are significantly different at $p < 0.05$.
[1:] Expressed of the fresh weight.
[2:] Expressed of the dry weight.

Table 4. Physicochemical composition of apricot jam elaborated with date syrups

Parameters	AJSMD	AJSDB
Water (%)[1]	29.55± 0.91 a	33.45 ± 0.85 b
Dry matter (%)[1]	70.45 ± 0.91 a	66.55 ± 0.85 b
pH	04.02 ± 0.00 a	03.86 ± 0.005 b
Titrable acidity (as citriq acid,%)[1]	0.91 ± 0.01 a	01.01 ± 0.00 b
Total soluble solids (°Brix)	62.00 ± 0.00 a	62.00 ± 0.00 a
Total sugars (%) [1]	54.52 ± 1.31 a	50.50 ± 1.82 b
Reducing sugar (%) [1]	25.24 ± 0.97 a	34.49 ± 0.92 b
Protein (%) [2]	0.46 ± 0.01 a	0.85 ± 0.02 b
Pectin (%) [1]	04.84 ± 0.28 a	04.15 ± 0.55 a
Ash (%) [2]	01.37 ± 0.04 a	01.66 ± 0.02 b
Potassium (mg/100g) [2]	620.23 ± 2.49 a	835.08 ± 1.59 b
Magnesium (mg/100g) [2]	35.32 ± 0.07 a	39.43 ± 0.05 b
Calcium (mg/100g) [2]	31.56 ± 1.47 a	23.74 ± 3.25 b
Copper (mg/100g) [2]	0.39 ± 0.01 a	0.70 ± 0.01 b

AJSMD: apricot jam, made with syrup of Mech-Degla.
AJSDB: apricot jam, made with syrup of Degla-Beida.
[1:] Expressed of the fresh weight.
[2:] Expressed of dry weight.
The different superscript letters within the same line are significantly different at $p< 0.05$.

Table 5. Jams Total polyphenols content and reducing power

Parameters	AJSMD	AJSDB
Total polyphenols[1]	122.20 ± 7.88 a	113.53 ± 8.97 a
Reducing power [2]	201.60 ± 12.67 a	185.32 ± 07.24 a

AJSMD: apricot jam, made with syrup of Mech-Degla.
AJSDB: apricot jam, made with syrup of Degla-Beida.
[1]: mg of equivalent gallic acid.
[2]: mg of equivalent ascorbic acid.
The different superscript letters within the same line are significantly different at $p< 0.05$.

Table 6. Prepared Jams color

Parameters	AJSMD	AJSDB
L^*	38.53 ± 0.20 a	40.80 ± 0.30 b
a^*	01.96 ± 0.15 a	03.53 ± 0.05 b
b^*	01.80 ± 0.10 a	04.56 ± 0.49 b

AJSMD: apricot jam, made with syrup of Mech-Degla.
AJSDB: apricot jam, made with syrup of Degla-Beida.
The different superscript letters within the same line are significantly different at $p < 0.05$.

Table 7. Sensory properties of apricot jams prepared using date syrups, compared to the commercial apricot jam

Product	Appearance	Texture	Taste	Color	Global acceptability
AJSDB	5.57 ±1.91 a	6.14 ± 1.56 a	5.14 ± 2.44 a	5.71 ±1.54 a	5.64 ± 1.88 a
AJSMD	7.35 ±1.33 ab	7.14 ±1.35 a	7.57 ± 1.08 b	6.7 ± 1.68 ab	7.19 ±1.66 b
AJS	6.35 ± 2.06 b	7.21 ±1.36 a	6.78 ± 1.62 b	7.28 ± 1.54 b	6.91 ± 1.38 b

AJSMD: apricot jam, made with syrup of Mech-Degla.
AJS: apricot jam prepared with sucrose (standard or commercial jam).
AJSDB: apricot jam, made with syrup of Degla-Beida.
The different superscript letters within the same columns are significantly different at $p < 0.05$.

The results of color analysis of apricot jam are given in Table 6. The clarity of jam (Degla-Beida syrup) was slightly higher than the jam (Mech-Degla syrup) preparation, with a significant difference ($p<0.05$). The same remarque is valuable for the parameters a* and b *, the observed differences are due to the original color of date syrups used and therefore the range of dates.

The results of sensory analysis of apricot jam are summarized in Table 7. Sensory analysis showed no significant difference between Mech-Degla syrup jam and the commercial jam for appearance, texture, taste and color (Table 7). All these results allowed us to classify the jam depending on the degree of consumer preference as follows: Mech-Degla jam, industrial jam then Degla-Beida jam. The substitution of sucrose by date syrup is possible up to 100 % in the case of Mech-Degla syrup. For jam (Degla-Beida syrup), it can be reproduced by the use of partial optimization replacement sucrose by date syrup in order to improve its organoleptic quality.

4. Conclusions

This study can bring added value to the common dates by the incorporation of date syrup instead of sucrose in the jams formulations. The apricot jam produced by date syrup was characterized by interesting nutritional value compared to jams made by sucrose: they are enriched with nutrients syrups dates as minerals, phenols and fiber (pectin). Sensory analysis indicated that apricot jam made by Mech-Degla syrup was most appreciated by the tasters and represents a competitive commercial jam. In the future, it would be interesting to characterize date syrups pectin and determine their rheological properties.

This work paves the way for manufacturers to produce this jam on an industrial scale and encourages farmers to save biodiversity of date varieties heritage.

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NON-ALCOHOLIC, NATURALLY-CARBONATED BEVERAGE FROM *DAUCUS CAROTA* USING *SACCHAROMYCES CEREVISAE* ISOLATE

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ABSTRACT

A pure yeast isolate from cheese whey was identified and characterized as *Saccharomyces cerevisae* strain NRRL Y-12632 by morphological, biochemical and 26S rRNA sequencing. This strain was used to produce non alcoholic naturally carbonated beverage from pure carrot (*Daucus carota*) juice and blended carrot-lemon juice (2:1), carrot-mint juice (2:1) and carrot-ginger juice (2:1). The juices were subjected to fermentation for 72 hrs. The physicochemical properties of 72 hrs fermented carrot:lemon beverage (2:1) were pH 3.15, TSS 12°B, CO_2 content 0.16 bar, acidity 0.41%, sugar-acid ratio 29.29% and alcohol 0.07% (v/v). The change in physicochemical properties was monitored after 15 and 30 days of refrigeration without adding any preservative. The changes in the physicochemical properties recorded for carrot-lemon beverage were pH 2.34, TSS 11°B, CO_2 content 0.21 bar, acidity 0.53%, sugar-acid ratio 20.75% and alcohol 0.14% (v/v) after storage for 30 days at 4°C without adding any preservative. Among the four beverages, carrot-lemon beverage (2:1) was highly acceptable when compared on 9 point hedonic scale. This technology can be a useful processing technology for perishable fruits and vegetables and can also help to overcome their seasonal overabundance.

1. Introduction

India is an agriculture based country which ranks second in fruits and vegetables production in the world, after China. As per National Horticulture Database-2012 published by National Horticulture Board during 2011-12, India produced 76.424 million metric tonnes of fruits and 156.33 million metric tonnes of vegetables. *Daucus carota* (Carrot) is one of the top-ten most economically important vegetable crops in the World (Simon et al., 2008) and India contributes 3,50,000 metric tons of the World's total carrot production (FAO, report 2006).

The rich carotenoid content of carrots not only helps to prevent oxidative damage inside our body. Studies have identified carrot to contain polyacetylenes as phytonutrients that can help inhibit the growth of colon cancer cells, especially when these polyacetylenes are found in their reduced (versus oxidized) form. Apart from the therapeutic value, consumers like carrot juice because of its high nutritive value, fiber, carbohydrates, vitamin A derived from its high α carotene (β ε-carotene), β-carotene content, color, aromatic compounds and refreshing characteristics (Desobry et al., 1998). However, due to seasonal excess of carrot, huge quantities go waste due to its perishable nature. A solution to this problem is the use of carrot juice for beverage production by fermentation. A beverage produced by fermentation process can be alcoholic or non-alcoholic. However, the measure of alcohol

content in beverage is important for quality and stability of the beverage, legal definition of categories, consumer tastes and information, taxation, product regulation and retail licensing. In traditional fermentation processes, natural microorganisms are employed in the preparation and preservation of different types of food. These processes add to the nutritive value of foods as well as enhance flavour and other desirable qualities associated with digestibility and edibility (Kolawole et al., 2007). For the production of fermented beverages, yeasts belonging to different genera are the microorganism of choice. Different yeast species are responsible for providing different concentration of alcohol, carbon dioxide, taste and fragrance to the beverages produced. However, with the general public becoming more and more conscious about the ill effects of consuming alcohol, the focus is now on the production of non-alcoholic or low alcoholic beverages from fruits and vegetables. Moreover, various fruits as well as condiments can be used as flavor enhancers by blending them with fruit juices during beverage production which also improves the nutritional value. In the present studies, production of beverage from carrot was carried out using a yeast isolate that was capable of producing low alcohol content and high carbonation.

2. Materials and methods

2.1.Isolation and purification of yeast isolates

Various sources including apple peels, grapes, resins, jaggery, rose petals, potato peel, lemon skin, cheese whey, carrot peel and orange rind were used for isolation of best suited strain of yeast which produced low alcohol concentration with high carbon dioxide production. Routine spread plating of appropriately diluted samples on Yeast Peptone Dextrose agar media containing chloramphenicol as an antibiotic was carried out. The cultures were incubated at 27± 1°C for 72 hours.

2.2. Screening of low alcohol, high carbonation producing yeast isolate

For the selection of low alcohol and high carbonation producing yeast, all the isolates (24 hrs old culture) were separately inoculated in carrot juice diluted with water (2:1). The brix of the juice was adjusted at 14ºB after pasteurization at 62.8ºC for 30 minutes. The physicochemical properties namely TSS (°B), carbondioxide content and alcohol content of beverages were studied after fermentation at 27±1 º C for 72 hours. On the basis of physicochemical properties of fermented beverages, best suited yeast was selected for beverage production. The strain was further characterized morphologically, biochemically and molecularly.

2.3. Characterization of yeast isolate

2.3.1.Morphological and biochemical characterization of yeast isolate

The colonies were studied for colour, shape, surface, margins and colony elevation on yeast peptone dextrose agar media according to Guillier (2003). The cultures were observed microscopically after staining in order to identify them morphologically. Yeast isolate was also biochemically characterized by fermentation test (Ghinea et al., 2009), urease test (Roberts et al., 1978), amylase production test (Fossi et al., 2009) and acid production test from glucose (Kurtzman et al., 2011).

2.3.2.Molecular characterization of yeast isolate

For molecular identification of the yeast isolate, its gDNA was isolated. Fragment of 26S rDNA gene was amplified by PCR from gDNA. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with DF and DR primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The 26S rDNA gene sequence was used to carry out BLAST with the nrdatabase of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple

alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4.

2.3.3.Production of non-alcoholic, naturally-carbonated beverage from carrot

Fresh, healthy and undamaged carrots, lemon, ginger and mint leaves were washed with chlorinated water and peeled. Juices of carrot, lemon, ginger and mint fresh leaves were extracted by electronic juicer and filtered with muslin cloth. Carrot juice was blended with lemon, mint or ginger juice. 2:1 ratio for blending was selected since higher dilutions with blends gave a bland taste to the beverage during lab trials while lower dilutions with lemon, mint or ginger gave too sour, too minty or too bitter taste respectively. This decision was also based on the outcomes of previous studies carried out in our lab where 2:1 ratio of *Citrus reticulate* and *Ananas comosus* with different blends were the most accepted beverages (Kaur et al., 2014). The blended juices were then diluted with autoclaved distilled water in ratio 1:2.

Pure and blended juices were pasteurized at 62.8°C for 30 minutes in 1 liter sterile glass bottles, cooled and brix was adjusted to 14°B by adding sugar solution (500g/l) followed by inoculation with 24 hr old culture of yeast isolate @ 0.5% (v/v). The bottles were incubated for 72 hrs at 27±2°C. The beverages were siphoned, bottled and stored under refrigerated conditions according to Jooyandeh (2013) and subjected to shelf life studies, microbiological assay and sensory evaluation.

2.3.4.Physicochemical and Microbiological analysis of fermented beverages

Physicochemical analysis of beverages was carried out by calculating the pH using digital pH meter, TSS (Total solid suspends) by using 0-32°B refractometer, carbon-dioxide content Buret Titration Method, alcohol content (Batac et al., 2012), total acidity (%citric acid by calculating according to Gaithersburg, 1999) and sugar-acid ratio after 3 days of incubation and 15 and 30 days of refrigeration. The beverages were evaluated on the basis of sensory scores using 9 point hedonic scale. Appropriately diluted beverages were spread-plated on YPD medium in order to check for any contamination.

2.4. Statistical analysis

Statistical analysis was done by using CPCS1 software developed by Maths, Statistics and Physics Department, PAU, Ludhiana, India. One-way and Two-way anova was applied and CD@5% was calculated.

3. Results and discussions

3.1. Screening of yeast isolate for beverage production

For the selection of low alcohol and high carbonation producing yeast, physicochemical properties namely TSS, carbondioxide content and alcohol content of beverages (Table 1) were determined after 72 hours of fermentation. Thereafter, the yeast isolate, initially designated as S10, was choosen for further beverage production as it produced low alcohol (0.107% v/v) with better carbonation (0.05 bar) as compared to others. All the 14 isolates showed an ability to produce very low amounts of alcohol even after 72 hours of fermentation. However, it was observed that the carbondioxide production ability of the strains was also low. This could be attributed to the fact that the samples were selected from sources which had lower probability of high alcohol producing yeasts.

3.2.Morphological, biochemical and molecular characterization

The colonies of isolate S10 (Fig.1a) exhibited viscous texture with off white coloration, round, small and smooth with even margins. After microscopic examination (Figure 1b), it was observed that the isolate S10 was oval in shape and showed active budding. When subjected to biochemical characterization, it was observed that isolate S10 had the capability to use glucose, galactose

and sucrose as source of carbon for fermentation. The isolate showed negative urease test, amylase production test and acid production test. When subjected to molecular characterization, a single discrete PCR amplicon band of 750 bp was observed when resolved on Agarose Gel (fig. 2). In gel image of 26S rDNA, lane 1 shows 26S rDNA amplicon band and lane 2 shows DNA marker. Consensus sequence of 668bp rDNA gene was generated from forward and reverse sequence data using aligner software. The isolate was characterized as *Saccharomyces cerevisiae* strain NRRL Y-12632 by 26S rRNA gene (GenBank Accession Number: JQ689017.1) based on nucleotide homology and phylogenetic analysis. The evolutionary history (Figure 3) was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 1.27150290 is shown. The evolutionary distances were computed using the Kimura 2-parameter method (Tamura et al., 2007) and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset. There were a total of 617 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Felsenstein, 1985).

3.3.Physicochemical analysis and shelf life study of fermented carrot beverage and its blends

Blended juices inoculated with isolate S10 were fermented for 72 hrs at 27±1°C siphoned, pasteurized and kept in refrigerator at 4°C for shelf life studies after 15 days and 30 days without adding any preservative. A change in the physiochemical properties of the carrot beverage (Table 2), carrot: lemon beverage (2:1) (Table 3), carrot: mint (2:1) beverage (Table 4) and carrot: ginger (2:1) beverage (Table 5) were studied since the acceptability and higher sensory score of the beverages are dependent on their physicochemical properties including pH, TSS, CO_2 content, % acidity and brix-acid ratio. Characteristic yeast colonies with no contamination were observed on yeast peptone dextrose agar media after microbiological assay of fermented carrot beverage as well as blended carrot beverages after 15 and 30 days of refrigeration. It was found that with an increase in number of days for which the beverage was held in refrigerated conditions, there was general trend of a decrease in brix, brix acid ratio (%), pH while there was an increase in CO_2, alcohol content (%) and total acidity. The increase in carbon dioxide content can be attributed to the fact that brix was decreasing due to utilization of sugar during fermentation as a result of which carbon dioxide was produced continuously as a by-product. Similar results were obtained by Jairath et al., (2012) who reported gradual increase in carbon dioxide content, total acidity (%citric acid), alcohol %(v/v) and gradual decrease in pH, TSS °B and brix acid ratio of low alcoholic self carbonated blended beverage of carrot and lemon.

3.4. Sensory evaluation of beverages

Sensory evaluation (Table 6) was done on the basis of 9-point hedonic scale by a group of 10 individuals after 15 and 30 days of refrigeration of the beverage. Blended beverage of carrot and lemon with the ratio of (2:1) was most accepted with an average score of 7.7 out of all the three beverages followed by carrot: ginger (2:1) beverage with average score of 7.

4. Conclusions

This technology can minimize the post harvest losses by storing the fruit in the form of beverage throughout the year with a lower inherent cost. Fruits like ginger and lemon can be made available for direct consumption in form of beverage as they are not palatable because of their bitterness, high acidity and astringency. This technology can be used for the production of varieties of low alcoholic self carbonated beverages with different flavours and aroma having high nutritional value. Moreover, it focuses on increasing shelf life of beverage without adding any preservative.

Table 1. Physicochemical properties of carrot beverage fermented (72hrs) by different isolates of yeast.

Sr. No.	Isolate used	CO_2 (bar)	Alcohol Content (%)	TSS (°B)
1	S1	0.011	0.012	9.5
2	S2	0.019	0.035	10.1
3	S3	0.027	0.042	10.3
4	S4	1.032	2.090	12.2
5	S5	0.024	0.039	11.7
6	S6	0.014	0.026	11.2
7	S7	0.035	0.103	08
8	S8	0.028	0.051	12.9
9	S9	0.012	0.021	11.6
10	S10	0.05	0.107	13.9

Table 2. Physicochemical properties of low alcoholic self carbonated carrot beverage.

Parameters	Time			F- ratio	CD (5%)
	3 Days	15 Days	30 Days		
pH	5.2	5.04	4.63	76.21	0.120
TSS, ^{0}B	14	12	11.5	524.49	0.200
CO_2, bar	0.03	0.07	0.08	1923.54	0.002
Total acidity (% citric acid)	0.08	0.18	0.20	36194.47	0.001
Brix- acid ratio (%)	168.26	67.04	56.15	-	NS
Alcohol % (v/v)	0.11	0.12	0.14	24.03	0.012

NS: Non significant

Table 3. Physicochemical properties of low-alcoholic self-carbonated carrot: lemon (2:1) blended beverage.

Parameters	Time			F-ratio	CD (5%)
	3 days	15 days	30 days		
pH	3.15	2.75	2.34	4898.77	0.020
TSS (^{0}B)	12	11	11	100.11	0.200
CO_2 (bar)	0.16	0.20	0.21	1936.77	0.002
Total acidity (% citric acid)	0.41	0.51	0.53	-	NS
Brix- acid ratio (%)	29.29	21.75	20.75	401767.50	0.025
Alcohol % (v/v)	0.07	0.13	0.14	4844.83	0.002

NS: Non significant

Table 4. Physicochemical properties of low-alcoholic self-carbonated blended carrot: mint (2:1) blended beverage.

Parameters	Time			F-ratio	CD (5%)
	3 Days	15 Days	30 Days		
pH	4.64	4.59	4.08	2904.77	0.020
TSS (^{0}B)	13	12	11.5	174.83	0.200
CO_2 (bar)	0.08	0.12	0.13	2161.81	0.002
Total acidity (% citric acid)	0.20	0.30	0.33	13823.13	0.002
Brix- acid ratio (%)	65.65	40	35.27	19190.30	0.409
Alcohol % (v/v)	0.07	0.12	0.15	5087.61	0.002

Table 5. Physicochemical properties of low-alcoholic self-carbonated carrot: ginger (2:1) blended beverage.

Parameters	Time			F-ratio	CD (5%)
	3 Days	15 Days	30 Days		
pH	5.36	5.16	5.05	747.08	0.020
TSS ^{0}B	14	11	11	900.95	0.200
CO_2 (bar)	0.04	0.07	0.11	3701.41	0.002
Total acidity (% citric acid)	0.10	0.18	0.29	-	NS
Brix- acid ratio (%)	136.72	61.38	38.19	-	0.072
Alcohol % (v/v)	0.08	0.08	0.17	7758.96	0.002

NS: Non significant

Table 6. Sensory evaluation of low -alcoholic self carbonated beverages produced from *Daucus carota.*

Sensory Attributes	Pure Carrot	Carrot:Lemon (2:1)	Carrot:Mint (2:1)	Carrot:Ginger (2:1)
1. Taste	6.0	7.9	5.9	6.6
2. Color	6.3	7.4	6.6	7.4
3. Aroma	5.8	7.6	6.7	7.2
4. Appearance	6.2	7.6	6.7	6.9
5. Mouth feel	5.7	7.4	5.9	6.6
6. Astringency	5.6	7.6	6.1	6.7
7. Flavor	5.3	8.0	6.0	7.1
8. Overall acceptability	5.9	7.9	6.2	7.3
9. Average value	5.9	7.7	6.3	7.0

*mean value of 10 replicates.

Nine Point hedonic scale : 9:like extremely, 8: like very much,7:like moderately, 6: like slightly, 5:neither like nor dislike, 4:dislike slightly, 3:dislike moderately, 2: dislike very much 1:dislike extremely.

	F-ratio	CD (5%)
Blends	1.12	NS
Attributes	1.41	NS
Blends x Attributes	3.76	1.23

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EFFECT OF COLD STORAGE ON THE QUALITY OF MINIMALLY PROCESSED CAULIFLOWER

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ABSTRACT
Cauliflower has been described as a vegetable with a high nutritional value due to its important content of vitamins, antioxidants and anti-carcinogenic compounds. Cauliflower inflorescences are harvested while they are totally immature, which implies severe changes in nutrient, water and hormonal status. Harvesting and the following processing can cause a severe stress determining the appearance of accelerated senescence symptoms. The effects of processing and cold storage of minimally processed green cauliflower were investigated. Florets were treated with antioxidants before storage in PE bags at 4°C for 21 days. During storage, weight loss, colour, firmness, soluble solids content and pH were evaluated. Minimally processed cauliflower showed good overall quality maintenance during cold storage but also susceptibility to browning of cut zones.

1. Introduction

Vegetables are important components of the diet, supplying a multitude of health-related phytochemicals. The consumption of cruciferous vegetables has a long tradition in many countries owing to their typical taste and qualities. Among these, cauliflower (*Brassica oleracea* L. var. *botrytis* L.) is well known throughout the world and is highly appreciated for its content of vitamin C (Lee and Kader, 2000), antioxidants and anti-carcinogenic compounds like glucosinolates. These are thioglycosides, characteristic of all Brassica species, that when degradated by either enzymes within the plant or within the alimentary tract, yield secondary compounds that have been linked to a reduced risk of specific forms of cancer. (Dillard and German, 2000; van Poppel et al., 1999; Verhoeven et al., 1997).

Cauliflower is cultivated for its inflorescences that are harvested while they are totally immature. There are varieties with different sizes and colours of the inflorescences (white, green, purple), different speeds of development (cycles which run for between 80 and 240 days) and different cultivation periods (from August to May in the case of Italy). Cauliflower is mainly commercialized as a fresh vegetable, but a minor part of the production is destined to freezing or fermentation. In recent years there has been a growing interest in an 'intermediate' form of commercialized cauliflower as a minimally processed product (Sanz et al., 2007).

Demand for fresh, minimally processed vegetables has led to an increase in the quantity and variety of products available to the consumer. Fresh-cut cauliflower should be considered as a new product and thus has received very little attention in the scientific literature. Cauliflower can be marketed cut in florets and packed in small units overwrapped with an appropriate film to avoid excessive

dehydration and the generation of inadequate atmospheres. This product must also be kept under refrigeration during marketing in order to maintain its quality and enhance its shelf life.

Quality and shelf-life of minimally processed vegetables may be affected by pre-harvest (Miceli and Miceli, 2014; Settanni et al, 2012; Settanni et al, 2013) or post-harvest (Miceli et al., 2014) factors, thus raw material quality, post-harvest treatments, packaging and storage conditions can greatly affect consumer acceptance and produce marketability.

The aim of the present work was to investigate the effect of processing and cold storage on minimally processed green cauliflower.

2. Materials and methods

Green cauliflower plants (*Brassica oleracea* L. var. *botrytis* L., 'Emaraude F1') were grown in the south-west coastal area of Sicily during autumn. Plants were selected for high quality and absence of defects. Curds were harvested in the second half of December and directly transported from the field to the laboratory. The cauliflower inflorescences were processed after manual removal of outer leaves by cutting into florets of 30-60 g each. Then they were washed by immersion for 5 min in tap water (added with 100 ppm free chlorine), rinsed to lower the free chlorine and finally dipped in a 1% citric acid solution for 5 min. Florets were drained and centrifuged using a handheld salad spinner to remove excess water and samples of 350 g were immediately placed in PE bags, sealed and stored at 4°C for 21 days. Immediately after processing and after 7, 14 and 21 days of storage, samples were randomly taken to evaluate the quality of minimally processed green cauliflower. Weight loss was evaluated by weighing samples soon after processing and during storage. The results were expressed as grams per 100 g of initial fresh weight.

Superficial colour of the top of the florets and of the cut zones was determined with a Colorimeter (Chroma Meter CR-400C, Minolta, Osaka, Japan), by measuring parameters L*, a* and b* at nine points on the florets and five point for the cut zone of each sample and storage time. Hue angle (h°) and Chroma (C*) was calculated as $h° = \arctan(b^*/a^*)$ when $a^* > 0$ and $b^* > 0$, or as $h° = 180° + \arctan(b^*/a^*)$ when $a^* < 0$ and $b > 0$ (McGuire, 1992) and $C^* = (a^{*2} + b^{*2})^{1/2}$.

Firmness was measured using a digital penetrometer (model 53205, TR Snc., Italy) with a 6 mm diameter stainless steel cylinder probe, in the cut zone of the stalk of 4 florets and in 6 points of the upper part for each sample.

Overall appearance was evaluated by an informal panel made of nine people (4 men and 5 women, aged 35-55) using a 1 to 5 scale, with 5 = excellent or having a freshly harvested appearance (e.g. green curds with no yellowing or riciness, free from handling defects and disease), 3 = fair/limit of marketability (e.g. some browning of the curds or the stalks, presence of minor defects or disease), and 1 = poor /unmarketable, with dark brown curds or stalks, and major defects and disease.

Samples of 150 g were then juiced with a commercial home juicer. The extract was centrifuged at 5000 rpm for 10 min and supernatant used to determine soluble solids content (SSC) and pH using respectively a digital refractometer (MTD-045nD, Three-In-One Enterprises Co., Ltd., Taiwan) and a digital pH-meter (waterproof pHTestr 30, Eutech Instruments, Nijkerk, The Netherlands).

All determinations were carried out in triplicate. Statistical analysis was performed using ANOVA and the means were separated using Duncan's Multiple Range Test.

3. Results and discussions

Minimally processed cauliflower was stored during 3 weeks at 4°C. During storage weight loss was very low and did not overcome 1% (table 1). This might be due to the low permeability to water vapour of the PE bags used for packaging, as found by Olarte et al. (2009) and Sanz et al. (2007) for broccoli and cauliflower.

Colour is one of the most important aspect in sensory evaluation. The surface colour of florets slightly changed after one week of storage and then remained stable for the other two weeks (table 2). Lightness value L* (represented by 0 = black and 100 = white) showed slight negative variations after 7 days with no other significant changes up to day 21. Coordinate a* decreased while coordinate b* increased after the first week of storage. The decrease of a* toward green indicates that there was no browning of the samples; the increase of b* indicates a yellowing of the samples. From these coordinates, Hue angle was calculated (90° = yellow and 180° = green). Florets showed initial Hue values of about 161 and did not significantly change after 21 days of storage. Chroma is a measure of colour purity or vividness. Chroma values significantly increased between days 0 and 7, after which levelled off. The stability of colour parameters of floret surface during cold storage of minimally processed green cauliflower may be due to a low variation of the contents of vitamin C, β-carotene and chlorophyll (Paradis et al., 1995). The colour of cut zones showed greater changes during cold storage. L* value decreased significantly after 7 d of storage and dropped continuously till the end of the storage period ($\Delta L_{0\text{-}21}$=16.2). The browning of cut zones was also evident in Chroma and Hue angle variations: colour saturation increased significantly during storage, while Hue angle values showed a progressive decrease until 14 days of storage.

Even though cauliflower is generally eaten cooked, the firmness of raw cauliflower is a good attribute of quality. It is related with curd compactness that decreases as inflorescence develops toward flowering. This modification can reduce shelf life and consumer acceptance of fresh or minimally processed cauliflower, as it is a good indicator of freshness or deterioration. The firmness of the upper part of curds before packaging was 37.6 N (table 1). During storage, this value slightly reduced and was statistically different only after 14 days of storage with no further change. Cold storage caused also a small but significant decrease of stalk hardness after 7 days of storage to a level maintained till the end of storage period.

During post-harvest the level of sugars of vegetables usually decreases since they are consumed by respiration while the supply of sugars from the plant is interrupted (Lemoine et al., 2009), nevertheless, soluble solids content (SSC) of minimally processed green cauliflower did not significantly varied during cold storage (table 1) as also found by Hodges et al. (2006).

The pH value of cauliflower (6.32 before packaging) slightly increased during the first 7 days of storage, after which no other changes occurred (table 1). This increase could be explained by the tissular breakdown that the vegetables undergo during storage; nevertheless, in the case of minimally processed vegetables, the capacity to respond physiologically is retained and cushions the variations in pH induced by the high levels of CO_2 which build up in the atmosphere inside the packs (Olarte et al., 2009).

Scores for overall appearance decreased slowly during storage (table 1). Florets maintained the highest score for the first week of storage. The attribute which suffered most during the second part of storage was the colour (browning) of the cut zones. This alteration of colour determined a reduction in overall appearance score (2.7) and negatively affected the marketability of minimally processed cauliflower at the end of the trial.

4. Conclusions

Cold storage is one of the most common technologies used to delay senescence, decay and quality loss during post-harvest life of vegetables. Fresh-cut vegetables suffer a severe processing stress that may greatly alter the physiology of tissues compared to intact organs (Lemoine et al., 2009). Thus, also minimally processed vegetables benefit of cold storage in order to keep quality throughout shelf life.

Green cauliflower showed to be suitable for processing as a minimally processed vegetable. Minimally processed green

cauliflower maintained a good firmness and sensory quality till 2 weeks of storage at 4°C.

Limitation of shelf life was due mainly to the browning occurred in the cut zones. The reduction of the colour alterations in the stalks may lead to prolong shelf life over 3 weeks.

Table 1. Influence of the storage at 4°C on quality parameters of minimally processed cauliflower.

Days at 4°C	Weight loss (%)	Firmness (N)		SSC (°Brix)	pH	Overall Quality
		Top	Stalk			
0		37.6a	66.8a	6.6	6.32b	5.0a
7	0.5b	35.1ab	63.3b	6.8	6.62a	4.8a
14	0.5b	33.1b	62.7b	6.5	6.58a	4.2b
21	0.8a	33.1b	61.3b	6.6	6.56a	2.7c

Data within a column followed by the same letter are not significantly different according to Duncan's multiple range test (P<0.05).

Table 2. Colour changes of floret tops and cut zones during storage at 4°C.

Days at 4°C	L*	a*	b*	Chroma	Hue angle
Floret top surface					
0	69.8a	-17.0a	50.0b	52.8b	161.3
7	65.6b	-20.3b	57.7a	61.2a	160.6
14	64.9b	-20.2b	57.1a	60.5a	160.6
21	64.1b	-19.0b	55.4a	58.5a	161.1
Cut zone					
0	80.8a	-3.9c	14.8c	15.3c	104.8a
7	70.7b	-2.7b	17.9b	18.1b	98.6b
14	68.3bc	-0.6a	20.6a	20.6a	91.7c
21	64.8c	-0.3a	22.5a	22.5a	90.8c

Data within a column followed by the same letter are not significantly different according to Duncan's multiple range test (P<0.05).

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ARTIFICIAL NEURAL NETWORK APPROACH TO PREDICT THE RHEOLOGICAL PARAMETER IN THE STUDY OF OIL SOLUTION DEGRADATION

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ABSTRACT

Study on vegetable oil has become more important for industrialists and academicians due to its wider usage in food industry, lubricant industry, biodiesel production, replacement for transformer oil and bio-polymer synthesis. Degradation tendency in mustard and sesame oil solution are studied using rheological property of oil added with CCl_4 at different concentrations. The rheological parameters specific, reduced and relative viscosity are studied for 16 samples using the laminar flow measurement. The oils are also heated up to four cycles at 200°C and their kinematic viscosity is also studied during each cycle of heating. The intrinsic viscosity of the oils are calculated using three model equations proposed by Solomon and Cuita, Ram Mohan Rao and Yaseen, and Deb and Chatterjee. The variation of viscosity with concentration is studied using new empirical equations and the r^2 values are found to be of good accuracy. Further analysis for accuracy and prediction of viscosity were obtained using Artificial Neural Networks (ANN) for various concentrations.

1.Introduction

Vegetable oils are complex mixtures of many triglycerides. A triglyceride is made up of three fatty acids attached to one glycerol molecule (Geller, 2000). One hundred grams of fat or oil yields 94% of fatty acids. Both the physical and chemical characteristics of fats are influenced greatly by the types and proportions of the fatty acids components (Rubalya, 2012) (Tong, 2002). Fatty acids are classified as saturated, monounsaturated (single double bond) and poly unsaturated (more than two double bond). The content of polyunsaturated fatty acids is directly proportional to the tendency of the polymer formation (Adolfo, 2006).

The degradation of oil from these sources is due to polymerization, hydrolysis and oxidation reactions. Heat not only serves as a catalyst, accelerating the formation of free fatty acids but it also initiates the polymerization of these oils (Isaac, 2004). The effect of overheating results in the conversion of polyunsaturated fatty acid components of the oil into saturated compounds, *trans* fats or other products of polymerization (Benedito, 2002). Polymerized degraded products are triacyl- glycerides, dimmers, oligomers, etc. (Hintze, 1993). The subsequent formation of polymers affects the viscosity of oil, which is the most important rheological parameter that influences the nature of the oil (Hui, 1999). Polymerization produces permanent marks in the fatty acid-cholesterol system of the human body. HDL-LDL ratio in the blood increases the overall blood cholesterol level. The accumulating cholesterol content in the blood gets lined up along the blood vessels, resulting in thickening and narrowing, a condition call “atherosclerosis” (Seniha, 2006) (Tony, 2007).

Viscosity of vegetable oils varies due to the oxidation reaction that takes place in the double

bond of fatty acids on different cycles of heating (Rubalya, 2013) (Abramovic, 1998). Oxidized oils are widely used in the manufacturing of oil based binders as it yields products having high viscosity which correlates with high strength, good stiffness, prolonged existence and environmental resistance (Seniha, 2006).

Artificial Neural Networks (ANN) is a simple computer program employed in a wide variety of fields like Computational Biology, Engineering, financial analysis etc. Neural Networks train themselves through experience of multiple iterations to gather knowledge using input-output relationships. The factors deciding the success of ANNs are the learning algorithm, architecture and its parameters. ANNs have been used to analyze and predict the viscosity of ionic liquids in a binary mixture and that of sucrose aqueous solutions with temperature and concentration (Wujian, 2002) (Salwani, 2008).

In the present study polymerization tendency of the sesame and mustard oil is analysed from the change in the rheological study. The findings of oil solution could be used in food industry, biopolymer, paint, ink, cosmetics etc. Hence the variations of relative, specific and reduced viscosity with concentration are studied using empirical equations and ANNs are used to predict random values with respect to concentration of the oil. Three theoretical model equations proposed by Solomon and Cuita, Ram Mohan Rao and Yaseen Deb and Chatterjee (Abdel-Azim, 1998) are used to study the intrinsic behavior of the oil.

2. Materials and methods

Sesame oil and mustard oil has been collected from a local grocery shop in Thanjavur, Tamilnadu, India. One hundred milliliters of unheated oil has been taken in a copper beaker and heated using electric device, stirring manually with glass rod. A microcontroller based temperature controller has been designed to control the sample temperature. To mimic the oil oxidation process during frying, the sample is exposed to four cycles of heating with the intervals of one hour to its frying temperature 200°C. The temperature is maintained by a temperature controller which has a very good accuracy of ± 2% of error. In order to ensure that the sample has been heated to the degradation conditions, it has been exposed to successive heating. 16 samples of oil solution were prepared by dissolving the oils (20ml, 40ml, 60ml and 80ml) at different concentration in CCl_4. So that total quantity is 100ml.

Viscosity of the oil was measured using Redwood Viscometer and Ostwald's Viscometer for oil solution by the ASTM D446 method. The *relative viscosity* (η_{rel}) of the sample is measured from the time that measures the rate of flow using the viscometer.

(η_{rel}) = viscosity of solution/viscosity of solvent (1)

3.Results and discussions

Mustard oil is one of the widely used vegetable cooking oils in North India containing 61% of monounsaturated fatty acids and 21% of polyunsaturated fatty acids. Sesame oil is predominantly used in South India, containing high potent antioxidants which are good for human health. Sesame oil has 40% of monounsaturated fatty acids and 41% of polyunsaturated fatty acids (Hui, 1999). There is not much research available which studies the effects of viscosity change due to polymerization of vegetable oil when it is heated to a high temperature near to frying conditions. Viscosity is the resistance offered to the flow of fluid from one part of the fluid moving relative to another one. Hence structural changes in the oil can be closely correlated with the viscosity (Fasina, 2008) (Anwar, 2007). Viscosity measurement is very much useful for obtaining behavioral and predictive information for product consistency and quality of the solution, and ultimately characterizing the solute molecules.

3.1.Variation of viscosity with time of heating

Figure 1 elucidates the variation of viscosity with time of heating. The viscosity of oil increases with time of heating as it was observed at room temperature where there is no thermal effect. The viscosity of oil is measured without adding any solvent. It was found that viscosity varies in a non-linear fashion with time and the r^2 values are 0.9895 for sesame and 0.9645 for mustard. The change in viscosity accompanying heating is due to aggregation of molecules together. Following aggregation, several chemical reactions takes place which links molecules into clusters and these molecular clusters are responsible for the increase in viscosity of the sample (Isaac, 2004). The increase in viscosity with further increase in the time of heating is 74% for mustard oil and 61% for sesame oil.

3.2. Relative Viscosity

Figure 2 illustrates the disparity of relative viscosity of unheated and heated mustard and sesame with concentration of oil solution in the X-axis. On thermal degradation of vegetable oil, free radicals from the initial phase gets attacked by the oxygen molecule, and oxidized to form free fatty acids. Also if the formed fat molecule radical collides with another fat molecule radical, Polymerization (Chain growth mechanism) stops. The solvent CCl_4 restricts the oxidization of radicals and prevents the fatty acid radicals from colliding with each other. From the above explanation it would be clear that the vegetable oil with relatively high percentage of unsaturated fats polymerizes quickly and easily. CCl_4 is used because it is a non-polar organic solvent and its viscosity is less. Relative viscosity is an index used to study the degree of polymerization. Polymers in oils arise by the formation of either carbon-carbon bonds or oxygen bridges between molecules (Hui, 1999). When an appreciable amount of polymer is present in oils, viscosity of oils also increases (Cristobal, 2007) (Erhan, 2002). Increased polymer formation tends to alter the cohesive and adhesive forces of the oil. Thus viscosity increases due to various interactions existing between the polymer molecules such as Van der Waal's forces, hydrophobic interactions, cross-linking etc. It is observed that the relative viscosities of heated oils are greater than the unheated oils but the percentage of increase is found to be more in mustard oil. This shows the tendency of formation of polymerization is more in heated mustard oil.

3.3. Specific Viscosity

Figure 3 shows the variation of specific viscosity of unheated and heated mustard and sesame oils with different concentration of oils dissolved in the solvent CCl_4. The specific viscosity is the relative viscosity of oil solution of known concentration minus 1; usually determined at low concentration of the polymer. Oxy-polymerization is an important method used for modification of triglycerides in oils. The ratio of saturated to unsaturated fatty acids will change due to degradation and polymerization of unsaturated fatty acids (Isaac, 2004; Benedito, 2002). The percentage of saturated and unsaturated fatty acids controls the viscosity of oils. The monounsaturated and polyunsaturated fatty acids in the oils get saturated by the substitution of hydrogen atom which makes the intermolecular binding stronger on heating to high temperature (Debora, 2013). The saturation of compounds in oils changes its viscosity. Those polyunsaturated fatty acids in mustard oil take part in polymerization comparatively more than monounsaturated fatty acids as specific viscosity shows nearly three times more variation as in Figure 3 (Rubalya, 2012).

3.4. Reduced Viscosity

Figure 4 shows the reduced viscosity changes with concentration of oil sample liquefied by the solvent CCl_4. From the relative viscosity, the specific viscosity was calculated and further the reduced viscosity was also calculated. Relative to this study, Polymer formation is well favoured by increase in temperature and increase in concentration. Reduced viscosity is also known as VISCOSITY NUMBER. There is a steep

increase in heated oils compared to unheated oils. The increase of reduced viscosity with growth in the concentration of oils is due to "molecular cluster" formation in oil solutions that are disordered on dilution leading to an increase in the hydrodynamic volume of the oil molecules. This is due to the formation of increased amounts of saturated composites (Balat, 2008). It is observed that polymerization tendency is more in mustard oil compared to sesame oil as the reduced viscosity of heated mustard oil get increase to two times the viscosity of unheated oils. On thermal degradation conversion of unsaturated fatty acids to triacylglycerols takes place in which at least one of the three fatty acyl chains is changed (Seniha, 2006; Shida, 2014).

3.5.Intrinsic Viscosity

Intrinsic viscosity is the contribution of the solute particles to the viscosity of the solution. Thus intrinsic viscosity calculation is a necessary when it comes to variation of oil characteristics with respect to solute concentration. In the current study, intrinsic viscosity is calculated using the three model equations proposed by Solomon and Cuita (Table 1), Ram Mohan Rao (Table 2) and Yaseen and Deb and Chatterjee (Table 3). The equations are as follows:

1) Solomon and Cuita

$$[\eta] = (2x\ (\eta_{sp} - \ln \eta_r))^{1/2}/c \quad (2)$$

2) Ram Mohan Rao And Yaseen

$$[\eta] = (\eta_{sp} + \ln \eta_r)/2c \quad (3)$$

3) Deb And Chatterjee

$$[\eta] = (3\ln \eta_r + 1.5\eta_{sp}^{\ 2} - 3\eta_{sp})^{1/3}/c \quad (4)$$

where: $[\eta]$ is the Intrinsic Viscosity of oil; $[\eta_r]$ is the Reduced Viscosity of oil; $[\eta_{sp}]$ is the Specific Viscosity of oil; c is the concentration of oil gm/L.

The calculated intrinsic viscosity using the 3 equations, do not vary much in the lower concentrations of oil. But as the concentration increases, all three equations start to give highly erratic values.

3.6.Inherent Viscosity

Figure 5 shows the variation of inherent viscosity with concentration of oil. The ratio between the natural logarithm of the relative viscosity and concentration is given by a term called the inherent viscosity. For pure solvents, relative viscosity is zero and varies directly with increase in concentration. Hence it can be used as an index for the presence of polymer in the solution. In case of non-zero concentration, the specific viscosity and inherent viscosities will vary even in case ideal solutions.

$$\eta_{inh} = \ln \eta_{rel}/c \quad (5)$$

where: η_{inh} is the Inherent viscosity of oil; η_{rel} is the Relative viscosity of oil; c is the concentration term.

3.7.Empirical equations

The variation of viscosity with concentration follows a non-linear pattern. Hence a nonlinear regression analysis method is used. The analyses are made to test the goodness of the fit. The regression analysis is done on the obtained values. Curve fitting is the process of formation of a mathematical function that agrees with the data points (Shida, 2014). Least square method of curve fitting is chosen for the analyses, and following two models are found to fit the most.

4.Reciprocal logarithm

$$\eta = 1/\ [A + B\ \ln(C)] \quad (6)$$

In the above equation A and B are constants, C is the concentration of the oil in the solution and η is the relative viscosity of the solution. The equation shows that relative viscosity is inversely proportional to the concentration of oil in the solvent. Table 4 shows the calculated constants A, B, standard error and regression coefficient for the viscosity variation in oil. It is observed that the value of A is related to the concentration of

CCl_4 in the solution. The increase in B value from unheated to heated may be due to increase in saturated fatty acids in the solution of heated mustard and sesame oil. The polymerization tendency of fatty acids increased on heating mustard oil. The regression coefficient shows a better fit with the observed data.

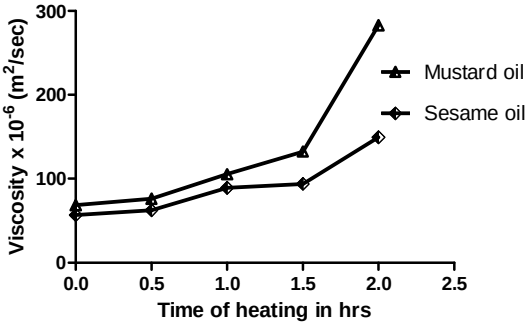

Figure 1. Variation of viscosity with time of heating

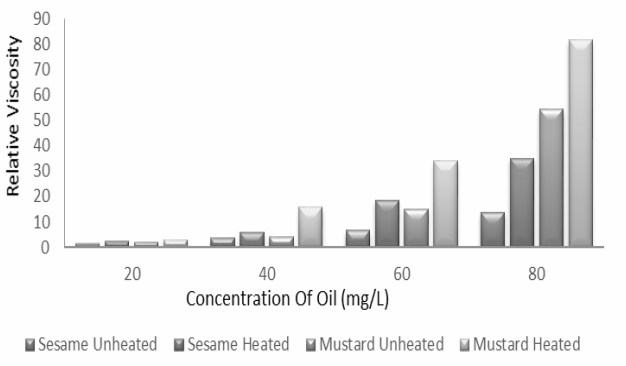

Figure 2. Variation of Relative Viscosity with respect to concentration of oil

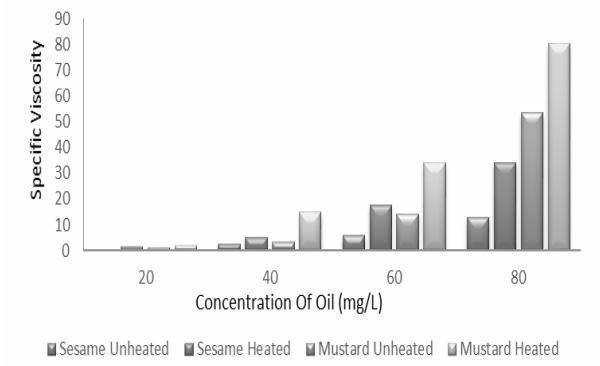

Figure 3. Variation of Specific Viscosity with respect to concentration of oil

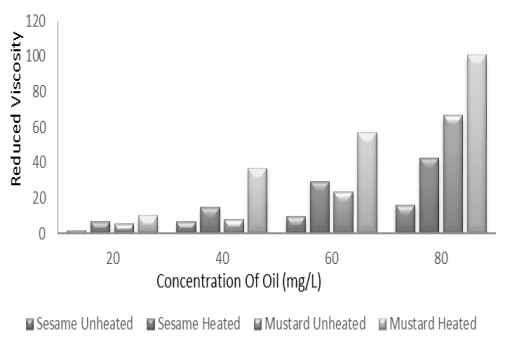

Figure 4. Variation of Reduced viscosity with respect to concentration of oil

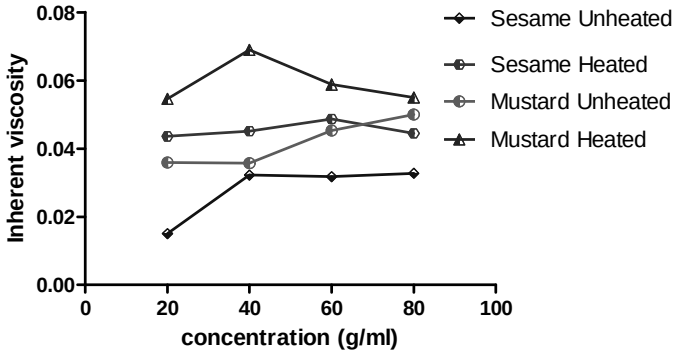

Figure 5. Variation of Inherent Viscosity with respect to concentration of oil.

Table 1.Intrinsic Viscosity of Sesame and Mustard Oils using equations from Solomon and Cuita

Concentration g/ml	Sesame Oil		Mustard Oil	
	Unheated	Heated	Unheated	Heated
20	1.58	5.08	4.09	6.67
40	4.09	6.39	4.67	12.25
60	4.60	9.02	7.98	13.00
80	5.63	9.75	12.42	15.42

Table 2. Intrinsic Viscosity of Sesame and Mustard Oils using equations from Ram Mohan Rao and Yaseen

Concentration g/ml	Sesame Oil		Mustard Oil	
	Unheated	Heated	Unheated	Heated
20	1.63	5.64	4.43	7.69
40	4.90	8.59	5.75	21.90
60	6.35	17.06	14.08	31.28
80	9.63	23.47	35.83	53.03

Table 3.Intrinsic Viscosity of Sesame and Mustard Oils using equations from Deb and Chatterjee

Concentration g/ml	Sesame Oil		Mustard Oil	
	Unheated	Heated	Unheated	Heated
20	1.63	5.50	4.36	7.39
40	4.63	7.66	5.36	16.56
60	5.58	12.46	10.73	19.66
80	7.48	14.75	20.04	26.45

Table 4. Regression with Reciprocal Logarithm Model

Parameters	Relative Viscosity of Sesame Oil η_r		Relative Viscosity of Mustard Oil η_r	
	Unheated	Heated	Unheated	Heated
A	0.867	1.621	3.072	0.088
B	1.035	1.079	1.042	1.615
r^2	0.989	0.994	0.985	0.999
Standard Error	1.36	1.11	1.08	0.64

Table 5. Regression with Modified Power Law Model

Parameters	Relative Viscosity of Sesame Oil η_r		Relative Viscosity of Mustard Oil η_r	
	Unheated	Heated	Unheated	Heated
A	1.362	0.581	0.297	0.088
B	-0.093	-0.127	-0.069	-0.195
r^2	0.992	0.983	0.987	0.997
Standard Error	2.16	2.48	2.2	2.53

Table 6. Prediction of relative viscosity using Artificial Neural Network (ANN)

Concentration g/ml	Relative Viscosity of Sesame Oil η_r		Relative Viscosity of Mustard Oil η_r	
	Unheated	Heated	Unheated	Heated
23	1.35	2.25	1.76	6.08
25	1.67	2.58	1.95	6.66
28	1.82	3.06	2.37	7.76
31	2.02	3.73	2.79	8.95
33	2.23	4.22	3.08	9.8
34	2.47	4.58	3.22	10.28
38	2.56	5.41	4.09	12.43
41	3.37	6.90	4.90	14.4
44	3.77	8.17	5.77	16.58
49	4.44	10.77	7.78	20.93
51	4.83	11.94	8.89	22.97
52	5.04	12.35	9.41	24.03

56	5.82	15.46	11.23	28.93
59	6.54	17.81	14.24	33.16
65	8.01	22.48	20.38	43.31
67	8.57	24.4	23.14	47.28
71	9.76	27.84	29.91	56.32
74	10.89	30.42	36.26	63.75
76	11.84	32.00	41.50	69.35

2.Modified Power law model

$$\eta = AB^C \quad (7)$$

In the equation A and B are constants, C is the concentration of oil in the solution and η is the relative viscosity of the solution. This equation can be written as ln η= ln A+ C ln B to study the variation of the empirical equation. The value of B increases more in mustard compared to sesame oil. Table 5 shows the value of the constants and the correlation co-efficient.

3.8.Artificial neural networks (ANN)

Variation of viscosity with concentration of oil is a useful parameter in determining the stability of the oil. Table 6 shows the predicted values of viscosity which are obtained by training a Neural Network with a sigmoidal function which is represented as:

$$Y = 1/(1 + e^{-x}) \quad (8)$$

In this case Y represents relative viscosity and x represents the concentration of oil. The sigmoid function is implemented for both input and output to train the neural network. The weights between output and the hidden layers are updated using the pseudo impedance control algorithm (Erhan, 2002). It is found that for using this rule, convergence is relatively faster than the original generalized delta rule. Before the execution of the Training process of Neural Network, the Input and Output parameters were normalized in the range of (-0.95; 0.95) via in order to acquire accurate results. The generalized delta rule uses sigmoid activation function f(x) = 1/ (1+e-x). The Back Propagation Algorithm is a supervised learning algorithm that aims at reducing overall system error to a minimum. In this learning procedure, an initial weight vector w0 is updated:

$$w_i (k+1) = w_i (k) + \mu (T_i - O_i) f'(w_i x_i) x_i \quad (9)$$

where: w_i is the weight matrix associated with i^{th} neuron; x_i is the input of the i^{th} neuron; O_i is the actual output of the i^{th} neuron; T_i is the target output of the i^{th} neuron; μ is the learning rate parameter.

The network has 2 input layers, 5 hidden layers and 1 output layer. Oil type and concentration is taken as input. 250 viscosity data points are taking for training pattern and 25 data points are taken for testing. The learning rate is 0.9.

Repeated usage of oil leads to degradation of oil quality which is not safe for any domestic purpose. Prediction of viscosity is one of the methods by which the safety of oil can be determined using pre-defined thresholds and various other parameters.

3.9.Statistical analysis

The measurement of viscosity is observed thrice and the standard deviation was premeditated using SPSS 12.0 software. The non-linear variation of viscosity with concentration of oil in dilute CCl_4 is studied and the regression coefficient is found to vary 0.905 to 1 gives accuracy of measurement.

4. Conclusions

Rheological parameters changes drastically with respect to heat exposures. The change in viscosity of oil in solution is due to molecular cluster formation and it was found to increase

with concentration. This shows more tendencies for polymerization due to chemical reactions when the oil is heated. The increase in viscosity for mustard oil is greater compared to sesame oil. This suggests that the formation rate of polymerizing compounds is more in Mustard oil and thus it has more tendencies to polymerize. The empirical equations fitted the experimental values in an accurate manner. The values of constants can be used to predict the viscosity of oils at different concentration. This analysis can be used in food oil quality control.

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EFFECTS OF DIFFERENT SOLVENTS ON THE EXTRACTION OF BIOACTIVE COMPOUNDS AND THEIR ACTIVITIES FROM *CASSIA SIAMEA* (LAMK.) LEAVES COLLECTED FROM DIFFERENT LOCATIONS

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Bioactive compound

ABSTRACT

The aim of this study is to evaluate bioactive compounds and antioxidant activities are present in *C. siamea* leaves collected from four different locations in and around Universiti Putra Malaysia using different solvents extractions: water, methanol, ethanol and ethyl acetate. The contents of ascorbic acid, α-tocopherol, *β*-carotene, tannin and total phenolic contents (TPC) were determined. Antioxidant activities were evaluated using DPPH radical scavenging assay, Ferric reducing antioxidant power (FRAP) and 2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS). The result showed that methanol extract indicated the highest value of the TPC, tannin contents, antioxidant activities (DPPH, FRAP and TEAC values) followed by the extracts of water, ethanol and ethyl acetate, respectively. TPC and tannins were found to be significantly ($p<0.05$) higher in methanolic extract of *C. siamea* leaves sample obtained from the location 3. Ascorbic acid content was found to be higher in water extract, whereas the ethyl acetate extract showed highest *β*-carotene contents (16.51-28.20 mg of *β*-carotene/g dry weight), irrespective of its locations obtained. DPPH and ABTS values of methanol extract were found to be significantly higher at location 3. On the basis of the results obtained, we confirmed that the methanolic extract of *C. siamea* leaves obtained from location 3 is a potential source of bioactive compounds rich in antioxidant activity.

1. Introduction

Consumption of various types of edible plant leaves in our daily diet provides excellent health benefits due to the rich source of various bioactive compounds (Veerachari & Bopaiah 2013). Higher intake of edible plant leaves has been associated with lower incidence of certain life threatening diseases such as cancer and cardiovascular diseases. The bioactive compound in the plant leaves includes polyphenols, flavonoids and vitamins (Harbaum et al., 2008; Sultana et al., 2009). Most plant polyphenols are reported to have antioxidant activities in both in vitro and in vivo (Jiang et al., 2008; Jimoh et al., 2007). Flavonoids are the group of plant polyphenols which includes flavones, chalcones and flavanols, prevents lipid peroxidation by various metal chelation and oxygen quenching mechanisms (Rice- Evans et al., 1995; 1996). Tocopherols and carotenoids have strong antioxidant activity, which protects the lipid peroxidation in foods and increases health benefits during consumption. Several traditional herbal leaves are well documented such as sage, oregano and rosemary due to the

extensive richness of phytochemicals (Kaliora et al., 2014). Leaves of lemon are rich in high concentration of functional compounds has been used in the treatment of digestive tract spasms. However, few traditional herbs such as *C. siamea* are little exploited. *C. siamea* is a native plant from Asian and African tropical countries widely used in various cuisines and in traditional medicines (Behemel & Neumann 1982). In Thailand, these flowers and leaves are highly used in cuisines for their typical flavor to their food. Purified extract of *C. siamea* leaves and flowers are rich in barakol, highly used as a laxative in Thai traditional medicine (Deachapunya et al., 2005). Bark extract is traditionally used as an antidiabetic agent. Root extract of these plants is also used for the treatment of various other diseases such as constipation and insomnia (Ahn et al., 1978). In addition it also has antibacterial effect due to the extensive richness of various phenolic compounds (Nakanishi et al., 1965).

The major bioactive compounds identified in *C. siamea* have been reported to be functional polyphenol (Deachapunya et al., 2005). However, no information is found in the literature regarding the extraction of functional compounds or total polyphenol in *C. siamea* leaves. Therefore, the purpose of this study was to extract the bioactive compounds present in *C. siamea* leaves collected from four different locations in and around Universiti Putra Malaysia using different solvents. Furthermore, the antioxidant activity of functional extract rich in antioxidants had been evaluated through the scavenging capacity of the DPPH radical scavenging assay, ferric reducing antioxidant power, and ABTS assay (2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid)).

2. Materials and methods

2.1 Chemicals and standards

Gallic acid, Folin-Ciocalteu reagent, Sodium tungstate, ortho-phosphoric acid, TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine), Ferric chloride hexahydrate ($FeCl_3.6H_2O$), DPPH, ABTS and BHT were obtained from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), α-tocopherol, *β*-carotene and ascorbic acid were purchased from Fisher (Pittsburgh, USA). Methanol, acetonitrile and other reagents used in this study were of analytical grade.

2.2 Materials

C. siamea leaves were collected from four different locations around Universiti Putra Malaysia (UPM) (Serdang, Selangor, Malaysia). The plant leaves were washed and cut into small pieces and freeze dried. The dried material was ground into a fine powder and passed through a 0.5 mm sieve, kept in an air-tight bottle at -25 ^{0}C until further extraction.

2.3 Extraction and yield of *C. siamea* leaves

The sample extraction was modified according to the method by Panidthananon (2008). The dried *C. siamea* leaves were solvent extracted for 90 min with water, methanol, ethanol and ethyl acetate respectively, in a mechanical shaker at room temperature. The extracts were then centrifuged at 4000 rpm for 30 min. The supernatant collected was filtered through No.1 filter paper and evaporated to dryness at 40 ^{0}C in a rotary evaporator. The dried sample of each extract was weighed to determine the yield of soluble compounds and stored in the freezer at -20 ^{0}C.

2.4 Determination of total phenolic contents

Total phenolic contents were determined according to the modified method of Maisuthisakul et al. (2007) using Folin-Ciocalteu reagent. Briefly, each extract was mixed with 1 ml of Folin-Ciocalteu reagent (before using diluted 10-fold with distilled water). The solution was mixed for 3 min and 0.8 ml of 7.5% (w/v) sodium carbonate solution was added. The mixtures were kept in the dark at room temperature for 2 hours. The absorbance of samples and blank were read at 765 nm. The total phenolic content was expressed as milligrams of gallic acid

equivalent per gram of dried sample (mg GAE/g dry weight).

2.5 Determination of tannin contents

The extraction of tannin content was carried out as described by Helrich (1990). 5 ml of sample extract was extracted with 10 ml of ether for 20 hours at room temperature. Ether was evaporated at 60 ^{0}C, 70 ml of distilled water was added and boiled for 2 hours. Allowed to cool and adjusted to a volume 100 ml by adding distilled water and filtered. The tannin content was determined using the colorimetric method at 760 nm. The tannin acid 0.1 mg/ml concentration was used as a standard.

2.6 Determination of ascorbic acid

Sample preparation was done according to the modified method of Abushita et al. (1997). 10 ml of the leaves extract was mixed with 10 ml of extraction solvent (0.3 M of meta-phosphoric acid and 1.4 M of acetic acid) in a flask. The ratio of leaf extract to extracting solvent was 1:1 (v/v). The flask was wrapped with aluminum foil to avoid the light and stirred for 15 min. The mixture solution was filtered through No.1 filter paper. Determination of ascorbic acid was done using high performance liquid chromatography (HPLC) method, C_{18}-reverse phase, 5mm of particle size, 250 mm length x 4 mm diameter (Waldbronn, Germany)**.** 3 mM Potassium dihydrogen phosphate in 0.35% (w/v) ortho-phosphoric acid was used as a mobile phase at a flow rate of 1 ml/min. Sample injection volume was 20 µl. The extract was filtered through a 0.45 µm of membrane filter before analysis. Calibration curve was obtained by using the stock solution of ascorbic acid dilution in 3% (w/v) meta-phosphoric acid in the range of 20-300 µg/ml for UV detection at 245 nm.

2.7 Determination of *β*-carotene contents

β-carotene was extracted according to the modified method modified by Chen et al. (2004). In brief, the leaf samples (20 ml) were mixed with 50 ml of extracting solution (hexane: acetone: ethanol, at a ratio of 70:15:15 (v/v/v)) including with 0.1% of magnesium carbonate ($MgCO_3$) and 0.05% of BHT. The mixture solution was stirred for 1 h in the dark at room temperature. Then, 30 ml of hexane was added, after mixing vigorously, and separated the upper layer. The extraction of lower layer was done twice, dried over sodium sulfate (Na_2SO_4) to adsorb trace of water and filtered through No.1 filter paper. The collected supernatant was evaporated in a rotary evaporator at 40 ^{0}C and redissolved in solvent mixing solution (methanol and methylene chloride in the ratio of 50:50 (v/v)). The extract was filtered through a 0.2 µm of membrane filter before HPLC analysis. *β*-carotene was analyzed using HPLC method as described by Lin & Chen (2003). The *β*-carotene contents were separated on C_{18}-reverse phase column 250x4.6 mm diameter, 5 µm of particle size. Acetonitrile, methanol and ethyl acetate (88:10:2) were used as a mobile phase at a flow rate of 1 ml/min. The sample injection volume was 20 µl and detected at 450 nm by UV-visible detector. The all-trans *β*-carotene concentration of 20-300 µg/ml was used as standard.

2.8 Determination of α-tocopherol contents

Sample preparation was done according to the modified method by Koning et al. (1996). Briefly, 10 ml of leaf extract was dissolved in 30 ml of water and added 21 g of KOH in 100 ml of ethanol. The 0.25 g of ascorbic acid was added to inhibit the oxidation and flushed with nitrogen. The saponification process was carried out by refluxing with KOH at 80 ^{0}C for 40 min and cooled down at room temperature; sample was first extracted with 300 ml of water and then extracted with 100 ml of N-Hexane/ethyl acetate (ration 9:1 (v/v)) 3 times. Each time, the organic solvent phase was collected and washed with 100 ml of distilled water until there was no reaction with phenolphthalein or no pink color with addition of phenolphthalein. The triplicates were combined and filtered through anhydrous

sodium sulfate (Na_2SO_4) and evaporated for 10 min by evaporator at 40 ^{0}C. The residues were redissolved in 20 ml of hexane, flushed with nitrogen and kept at -25 ^{0}C until analysis. The α-tocopherol content was determined by HPLC method using C_{18} reverse phase column, 5 µm of particle size, 250x4.6 mm, with methanol/water as the mobile phase at a ratio 94:6 (v/v) and flow rate at 1 ml/min. The UV detection wavelength was measured at 292 nm. The concentration of α-tocopherol standard solution was prepared in the range of 20-200 µg/ml (R^2 = 0.998). The results were expressed in mg/l of α-tocopherol per gram dry weight.

2.9 Determination of antioxidant activities

2.9.1 DPPH radical scavenging assay

DPPH radical scavenging assay was carried out as described by Ao et al. (2008) with some modifications. 100 µl of the extract (100, 200, 300, 400, 500 and 1,000 µg/ml) was added to 3.9 ml of a 60 µM DPPH solution in 95% ethanol. The standard ascorbic acid was used for a positive control. The absorbance at 517 nm was measured after 30 min. incubation in the dark. The percentage inhibition (%) of DPPH was calculated as [(Ao-Ae)/Ao] x 100 (Ao = the absorbance of the blank, Ae = the absorbance of the sample extract). The 50% of inhibitory DPPH radical concentration (IC_{50}) for the extract was calculated by plotting the inhibition curve in linear range versus the sample concentration.

2.9.2 Ferric reducing antioxidant power assay

The FRAP assay was carried out according to the modified method of Panidthananon (2008). Ferrous sulfate ($FeSO_4.7H_2O$) solution in de-ionized water (0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2 mM) was prepared for standard. The stock solution of FARP reagent was freshly prepared by mixing 5 ml of 20 mM $FeCl_3.6H_2O$, 5 ml of 10 mM TPTZ solution in 40 mM HCl and 50 ml of 300 mM sodium acetate buffer pH 3.6 at a ratio of 1:1:10 (v/v/v) and allowed to warm at 37 ^{0}C. The reaction mixture contained 1,000 µl of FRAP solution, 500 µl of de-ionized water and 100 µl of sample extracts. The standard solution and control were added to the same test tubes. The absorbance at 593 nm was measured in 4 min at room temperature. Determination of the FRAP values were calculated from a standard curve of $FeSO_4.7H_2O$ linear equation. The data were expressed as mM of ferrous sulfate per gram dry weight.

2.9.3 ABTS assay

ABTS was carried out according to the modified method of Panidthananon (2008). The ABTS solution was prepared by mixing 7 mM $ABTS^{\bullet+}$ solution with potassium persulfate solution and the mixed solution was kept for 12 h in the dark at room temperature. The solution was diluted by mixing 1 ml of $ABTS^{\bullet+}$ solution with 89 ml of ethanol to obtain the range an absorbance of 0.70±0.02 units at 734 nm. The leaf extract 100 µl was mixed with 3 ml of $ABTS^{\bullet+}$ solution and incubated for 6 min in the dark at room temperature before reading absorbance at 734 nm. Trolox solution (0.1-2.5 mM) was used as standard. The TEAC values were expressed in mM of Trolox equivalence (TE) per gram dry weight.

2.9.4 Statistical Analysis

Data was analyzed using Minitab Program Version 16.0. Analysis of variance (ANOVA) and Turkey's test were performed to compare significant differences between samples. The confidence limit was at $p<0.05$. The results were obtained at least in triplicates and expressed as mean ± standard deviation (SD).

3. Results and discussions

3.1 Total extraction yield, total phenolic contents, and tannin contents

Total extraction yield, total phenolic contents, and tannin contents of different solvent extracts of *C. siamea* leaves collected from four different locations are presented in Table 1 and Figure 1. Methanol and water extracts showed significantly higher extraction yield of the extracts than ethanol and ethyl

acetate (p<0.05), irrespective of the locations obtained. These solvent extracts differed in different values of extraction because they had been different in polarity and also possessed different extractability of bioactive compounds (Marinova & Yanishlieva, 1997). This indicated that highly polar extraction solvents, such as water and methanol, had been more effective than less polar solvents, such as ethanol and ethyl acetate in extracting hydrophilic bioactive compounds or phenolic antioxidants from *C. siamea* leaves. In fact, some researchers have also reported that the high extraction yield contained high antioxidant activities and phenolic substances of various plant extracts (Guillen & Mazanos 1996; Lehtinen & Laakso 1998; Borneley & Peyrat-Maillard 2000). TPC of the *C. siamea* leaves showed significantly the highest in methanol extracts (p<0.05), followed by water, ethanol, and ethyl acetate, respectively. However, different locations showed different TPC based on the solvents used in the extraction (Table 1). The TPC among the extracts ranged from 11.18 to 472.03 mg GAE/g dry weight and methanol extracts of location 3 had the highest value of TPC (p<0.05) with concentration of 472.03 mg GAE/g dry weight. Kaur et al. (2006) also reported that the ethanol extract of *C. siamea* flowers had total phenolics of 257 mg/g GAE and that TPC was related to antioxidant activity. Chanwitheesuk et al. (2005) also reported that methanol extract of *C. siamea* leaves showed high total phenolic content of 384 mg GAE/g dry weight, while Maisuthisakul et al. (2008) reported about *C. siamea* flower extracted with 95% of ethanol had a total phenolic value of 51.7 mg GAE/g dry basis. Higher TPC found in the leaves had been mostly due to the higher content of polyphenolic compounds in those leaves. On the other hand, the tannin contents of *C. siamea* leaves were in the range of 9.73 to 219.47 mg tannic acid/g dry weight and the results are shown in Table 1 and Figure 1. There were significant differences among the various solvent extractions and locations (p<0.05). Methanol extracts at location 3 showed the highest tannin contents (219.47 mg tannic acid/g dry weight), followed by water, ethanol, and ethyl acetate, respectively. Thus, it confirmed that location and extraction solvents highly affected the yield, TPC, and tannin contents of *C. siamea* leaves extract. Chanwitheesuk et al. (2005) also reported that methanol extract of *C. siamea* leaves had tannin contents of 110 mg of tannic acid/g dry weight. Tannins are polyphenols with high molecular weight that can be divided into two parts: condensed and hydrolysable tannins. Isolation of tannins by using alcoholic extract from *Phyllanthus amaruss* found that tannins had potential antioxidant activity (Ujwala et al., 2012). Based on the study, the high yield was compatible with the high total phenolic contents, tannin contents, and also antioxidant activities.

3.2 Total ascorbic acid, *β*-carotene, and α-tocopherol contents

Ascorbic acid, *β*-carotene, and α-tocopherol contents of different solvent extracts of *C. siamea* leaves collected from four different locations are presented in Table 2 and Figure 2. The water extracts had the highest concentration of ascorbic acid that varied from 6.59 to 9.43 mg of ascorbic acid/g dry weight, but there was no significant difference among locations. Water extracts showed significantly (p<0.05) higher ascorbic acid contents than methanol, ethanol, and ethyl acetate, respectively, because ascorbic acid is a water soluble substance, so all water extracts had higher ascorbic acid than other solvents. Higher water soluble ascorbic acid contents were also found in the flowers of *C. siamea*. According to Maisuthisakul et al. (2008), *C. siamea* flowers had a high content of ascorbic acid of 4.83 mg/g for dry basis. High amount of ascorbic acid was also found in other leaves, includes pumpkin leaves (1.71 mg/g dry weight) and black pepper leaves (1.81 mg/g dry weight) (Ogunlesi et al., 2010).

On the other hand, *β*-carotene contents of *C. siamea* leaves were in the range of 11.14 to 28.20 mg of *β*-carotene/g dry weight, as

presented in Table 2 and Figure 2. The *β*-carotene contents of ethyl acetate extracts were the highest, which ranged from 14.21 to 28.20 mg of *β*-carotene/g dry weight with significant differences among locations ($p<0.05$). The *β*-carotene contents of methanol and ethanol extracts gave rather similar results between four locations and did not show significant differences ($p>0.05$). However, extracts from location 4 showed higher *β*-carotene contents for both ethanol and ethyl acetate extracts. Besides, higher level of carotenoid is reported in flowers and fruits of most tropical plants, which are responsible for the pigment of red or orange color and antioxidant activity (Segantini et al., 2012). The amount of α-tocopherol contents of ethyl acetate extracts showed the highest values of all extracts, followed by ethanol and methanol extracts, respectively. α-tocopherol contents of *C. siamea* leaves were in the range of 0.75 to 1.12 mg /g dry weight, as presented in Table 2 and Figure 2. The amount of α-tocopherol contents of methanol, ethanol, and ethyl acetate extracts indicated no significant difference between the locations and no significant difference between the extracts in locations 1, 2, and 4 ($p>0.05$). Higher amount of α-tocopherol contents had been found in ethyl acetate extracts at location 3. Based on the study, the ascorbic acid and α-tocopherol contents showed insignificant difference between locations as there was no difference in variety and growing conditions.

3.3 Antioxidant activities

The results of DPPH (IC_{50} values) are presented in Table 3 and Figure 3(a). The lower amount of IC_{50} indicated higher antioxidant activity. However, %DPPH of inhibition, FRAP, and TEAC values inverted with IC_{50} with their higher values indicated higher antioxidant activity. The IC_{50} values of the methanol extracts containing the highest amount of TPC was found to be most active scavenger, followed by water, ethanol, and ethyl acetate extracts. There were significant differences in the IC_{50} values among the extracts and the locations ($p<0.05$). The methanol extracts of the sample leaves at location 3 was significantly ($p<0.05$) the highest in IC_{50} values of 204.56 μg/ml, follow by water, ethanol, and ethyl acetate extracts, whereby the IC_{50} values were 292.57, 758.28, and 15,535.10 μg/ml, respectively. The IC_{50} values of ethyl acetate extracts were very low (>15,000 μg/ml); therefore, they were not presented in Figure 3(a).

Furthermore, the presence of antioxidant compounds diminished the DPPH free radicals absorption maximum at 517 nm, by reducing its hydrazine form either by donating hydrogen atom or electron. The DPPH inhibition of *C. siamea* leaves is expressed in percentage of various solvent extracts collected from different locations. *C. siamea* leaves exhibited strong free radical scavenging activity on DPPH assay, respective of its solvents and location. The %DPPH of inhibition is shown in Table 3 and Figure 3(b). The results revealed that of all the solvent extracts, methanol extracts showed significantly the highest %DPPH of inhibition of 66.22% at concentration of 300 μg/ml ($p<0.05$). Higher %DPPH of inhibition was observed at location 3. The higher radical scavenging %DPPH of inhibition had been associated with the lower IC_{50} values. Kaur & Arora (2011) also reported that leaves extract of *C. siamea* by using various solvents showed relatively lower antioxidant activity (25% to 50% of DPPH inhibition at concentration of 1000 μg/ml) than bark extract (60.5% of DPPH inhibition at concentration of 800 μg/ml). Similarly, Kaur et al. (2006) also reported that the neutralized extract of *C. siamea* flowers by using ethanol extract showed higher antioxidant activity about 96% of DPPH at concentration of 250 μg/ml. Besides, ferric reducing antioxidant power (FRAP) assay was used to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion in the extracts from *C. siamea* leaves. In the assay, the presence of reducers (antioxidants) in *C. siamea* leaves caused the reduction of Fe^{3+} in the extracts to the blue coloured-Fe^{2+}, depending on the reducing power of each extract obtained from different locations.

Table 1. Total extraction yields, total phenolic contents, and tannin contents of different solvent extracts of *C. siamea* leaves (mean* ± SD[b])

Locations	Solvent extracts			
	Water	**Methanol**	**Ethanol**	**Ethyl acetate**
Total extraction yields (%)				
Location 1	34.18 ± 1.00^{NS,a}	32.39 ± 1.06^{AB,a}	11.55 ± 1.07^{BC,b}	3.50 ± 0.46^{B,c}
Location 2	33.50 ± 1.17 ^{a}	33.36 ± 1.05^{AB,a}	10.68 ± 1.01^{C,b}	2.45 ± 0.20^{C,c}
Location 3	34.53 ± 1.30 ^{a}	34.83 ± 1.08^{A,a}	14.96 ± 0.99^{A,b}	3.15 ± 0.14^{BC,c}
Location 4	32.33 ± 1.12 ^{a}	31.95 ± 0.99^{B,a}	13.03 ± 0.18^{AB,b}	4.94 ± 0.45^{A,c}
Total phenolic contents (mg GAE/mg dw)				
Location 1	317.86 ± 9.89^{A,b}	416.38 ± 10.63^{C,a}	134.90 ± 5.12^{B,c}	17.21 ± 0.26^{A,d}
Location 2	318.02 ± 8.85^{A,b}	437.37 ± 3.23^{B,a}	133.47 ± 5.64^{B,c}	11.18 ± 0.34^{D,d}
Location 3	321.37 ± 8.44^{A,b}	472.03 ± 2.62^{A,a}	163.34 ± 4.84^{A,c}	13.57 ± 0.79^{C,d}
Location 4	297.76 ± 8.61^{B,a}	301.11 ± 7.63^{D,a}	107.78 ± 1.33^{C,b}	15.82 ± 0.65^{B,c}
Tannin contents (mg tannic acid/g dw)				
Location 1	195.13 ± 1.05^{A,a}	185.75 ± 4.46^{C,b}	42.09 ± 3.35^{C,c}	13.50 ± 0.80^{A,d}
Location 2	163.65 ± 1.05^{B,b}	206.74 ± 5.75^{B,a}	71.27 ± 6.20^{A,c}	9.73 ± 0.43^{C,d}
Location 3	151.72 ± 0.52^{C,b}	219.47 ± 5.91^{A,a}	56.92 ± 9.72^{B,c}	11.68 ± 0.49^{B,d}
Location 4	143.13 ± 2.61^{D,a}	112.60 ± 3.41^{D,b}	47.83 ± 5.27^{BC,c}	10.12± 1.10^{C,d}

*Values are means, [b]SD are standard deviation, the data presented as mean ± SD with different capital letters (A-D) within the same column and mean ± SD with different small letters (a-d) within the same row, are significant differences (p<0.05) ; NS = not significant among locations (p>0.05) and ns = not significant among solvents (p>0.05).

Table 2. Ascorbic acid, *β*-carotene, and α-tocopherol contents of different solvent extracts of *C. siamea* leaves (mean* ± SD[b])

Locations	Solvent extracts			
	Water	**Methanol**	**Ethanol**	**Ethyl acetate**
Ascorbic acid contents (mg ascorbic acid/g dw)				
Location 1	9.43 ± 1.96^{NS,a}	1.84 ± 0.03^{NS,b}	1.50 ± 0.15^{NS,b}	0.81 ± 0.01^{NS,b}
Location 2	8.96 ± 0.23 ^{a}	2.20 ± 0.82 ^{b}	2.06 ± 0.69 ^{b}	0.83 ± 0.01^{b}
Location 3	8.37 ± 1.73 ^{a}	2.35 ± 0.95 ^{b}	1.78 ± 0.08 ^{b}	0.83 ± 0.01^{b}
Location 4	6.59 ± 0.39 ^{a}	2.25 ± 0.24 ^{b}	1.48 ± 0.23 ^{c}	0.95 ± 0.10^{c}
***β*-carotene contents (mg of *β*-carotene/g dw)**				

Location 1	ND	13.95 ± 0.60^{NS,ns}	12.70 ± 3.17^{B}	16.51 ± 1.84^{B}
Location 2	ND	11.30 ± 3.89 ^{ns}	11.14 ± 1.48^{B}	14.21 ± 2.09^{B}
Location 3	ND	12.05 ± 2.11 ^{ns}	13.39 ± 0.62^{B}	14.33 ± 3.26^{B}
Location 4	ND	11.74 ± 0.84 ^{c}	19.62 ± 0.95^{A,b}	28.20 ± 1.41^{A,a}
α-Tocopherol contents (mg α-tocopherol/g dw)				
Location 1	ND	0.84 ± 0.08^{NS,ns}	0.91 ± 0.15^{NS}	0.92 ± 0.21^{NS}
Location 2	ND	0.75 ± 0.04 ^{ns}	0.93 ± 0.38	0.95 ± 0.12
Location 3	ND	0.82 ± 0.10 ^{b}	0.87 ± 0.05 ^{b}	1.12 ± 0.12 ^{a}
Location 4	ND	0.76 ± 0.04 ^{ns}	0.91 ± 0.25	0.97 ± 0.06

*Values are means, ^{b}SD are standard deviation, the data presented as mean ± SD with different capital letters (A-D) within the same column and mean ± SD with small letters (a-d) within the same row, are significant differences (p<0.05) ; NS = not significant among locations (p>0.05) and ns = not significant among solvents (p>0.05).

Table 3. The antioxidant activities of different solvent extracts of *C. siamea* leaves (mean* ± SD^{b})

Locations	**Solvent extracts**			
	Water	**Methanol**	**Ethanol**	**Ethyl acetate**
DPPH (IC_{50}, µg/ml)				
Location 1	367.47 ± 1.89^{B,c}	239.18 ± 1.06^{B,d}	913.81 ± 4.51^{B,b}	15,694.09 ± 9.08^{C,a}
Location 2	311.44 ± 1.04^{C,c}	223.24 ± 5.05^{C,d}	897.11 ± 4.23^{C,b}	16,502.45 ± 50.34^{B,a}
Location 3	292.57 ± 1.03^{D,c}	204.56 ± 3.38^{D,d}	758.28 ± 4.97^{D,b}	15,535.10 ± 40.02^{C,a}
Location 4	415.16 ± 0.94^{A,c}	384.45 ± 3.80^{A,c}	1345.05 ± 8.98^{A,b}	20,296.04 ± 138.70^{A,a}
% DPPH inhibition (300 µg/ml)				
Location 1	43.85 ± 1.15^{C,b}	60.31 ± 0.81^{C,a}	19.68 ± 1.05^{B,c}	1.60 ± 0.53^{B,d}
Location 2	51.83 ± 0.67^{B,b}	63.52 ± 1.08^{B,a}	19.85 ± 1.03^{B,c}	3.01 ± 0.17^{A,d}
Location 3	59.95 ± 0.95^{A,b}	66.22 ± 1.02^{A,a}	23.14 ± 0.42^{A,c}	3.05 ± 0.14^{A,d}
Location 4	39.40 ± 0.67^{D,b}	41.44 ± 0.78^{D,a}	13.11 ± 0.50^{C,c}	2.01 ± 0.29^{B,d}
FRAP values (mM $FeSO_4$/g dw)				
Location 1	7.80 ± 0.22^{B,b}	13.20 ± 1.45^{A,a}	5.34 ± 0.55^{B,c}	0.19 ± 0.06^{NS,d}
Location 2	7.45 ± 0.89^{B,b}	11.90 ± 1.42^{A,a}	5.79 ± 0.14^{AB,c}	0.12 ± 0.03 ^{d}
Location 3	8.85 ± 0.40^{A,b}	12.09 ± 2.28^{A,a}	6.08 ± 0.15^{A,c}	0.21 ± 0.19 ^{d}
Location 4	7.17 ± 0.69^{B,a}	6.71 ± 0.77^{B,a}	4.44 ± 0.13^{C,b}	0.16 ± 0.08 ^{c}
TEAC values (mM Trolox/g dw)				
Location 1	9.25 ± 0.73^{A,b}	11.95 ± 0.29^{B,a}	3.29 ± 0.01^{NS,c}	0.40 ± 0.04^{B,d}
Location 2	7.94 ± 0.39^{B,b}	11.50 ± 0.66^{B,a}	3.35 ± 0.02 ^{c}	0.11 ± 0.01^{D,d}
Location 3	6.94 ± 0.16^{C,b}	13.24 ± 1.21^{A,a}	3.74 ± 0.90 ^{c}	0.22 ± 0.02^{C,d}
Location 4	8.52 ± 0.26^{B,a}	5.42 ± 0.33^{C,b}	3.31 ± 0.02 ^{c}	0.70 ± 0.06^{A,d}

*Values are means, ^{b}SD are standard deviation, the data presented as mean ± SD with different capital letters (A-D) within the same column and mean ± SD with small letters (a-d) within the same row, are significant differences (p<0.05) ; NS = not significant among locations (p>0.05) and ns = not significant among solvents (p>0.05).

Table 4. Correlation coefficients of total phenolic, tannin, α-tocopherol, ascorbic acid and *β*-carotene contents with the antioxidant activity assays

Antioxidant activity assays	**TPC**	**TC**	**tocopherol**	**ascorbic acid**	**carotene**
% DPPH	0.986	0.965	-	0.870	-
FRAP	0.963	0.922	-	0.848	-
TEAC	0.979	0.977	-	0.796	-
Average	0.976	0.955	-	0.838	-

TPC: Total phenolic contents (mg GAE/g dw); TC: Tannin contents (mg tannin/g dw); FRAP: Ferric reducing antioxidant power (mM $FeSO_4$/g dw), ABTS (TEAC values): 2,2/-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (mM Trolox/g dw), DPPH: 2,2-diphenyl-1-picrylhydrazyl, Ascorbic acid (mg of ascorbic acid/g dw), *β*-carotene (mg of *β*-carotene/g dw), α-tocopherol (mg of α-tocopherol/g dw).

The FRAP value of *C. siamea* leaves was in the range of 0.12 to 13.20 mM $FeSO_4$ /g dry weight, as shown in Table 3 and Figure 3(c). On the other hand, the FRAP values of methanol extract of *C. siamea* leaves had significantly (p<0.05) higher reducing power than other solvents, followed by water, ethanol, and ethyl acetate, respectively. Kaisoon et al. (2011) also reported that *C. siamea* flowers had higher reducing power activity. They found that the soluble and bound extracts of *C. siamea* flowers had FRAP values of 0.073 and 0.266 mM $FeSO_4$/g dry weight, respectively. Free radical scavenging activity of different solvent extracts of *C. siamea* leaves collected from different locations was also tested by their ability to bleach the stable ABTS radicals, and the data are presented in Table 3 and Figure 3(d). Meanwhile, the TEAC value from ABTS assay of *C. siamea* leaves was in the range of 0.11 to 13.24 mM Trolox equivalent/g dry weight. The TEAC values of methanol extracts were the highest, followed by water, ethanol, and ethyl acetate extracts, respectively, and the methanol extract of the sample leaves at location 3 showed higher TEAC value (13.24 mM Trolox equivalent/g dry weight) than other locations. It was highly correlated with the TPC and DPPH activities of methanol extract of *C. siamea* leaves at location 3. Similarly, Phomkaivon & Areekul (2009) also reported that ethanol extract of *C. siamea* flowers by using 80% of ethanol, the TEAC values showed higher ABTS radical scavenging activity of 4.55 mM Trolox equivalent/g dry basis, which is similar to the TEAC values retrieved in this study.

In addition, the results of antioxidant activities from *C. siamea* leaves extracts showed that methanol extracts exhibited the highest antioxidant activities, followed by extracts of water, ethanol, and ethyl acetate respectively. These results are in agreement with Yang et al. (2006), who extracted 150 species of edible plants and compared the results between using methanol and water extracts. For 133 vegetable plants, the methanol extracts had greater antioxidant activity for TEAC values than water extracts and the shoot of *C. siamea* was included as one with very high level of TEAC values and superoxide scavenging activity. In this study, it can be confirmed that the polyphenol contents were related to antioxidant activities. The higher TPC in the extracts indicated that there were high antioxidant activities. This agreed to the report of Maisuthisakul et al. (2008), which stated that plants with high contents of phenolics and flavonoids had high antioxidant activities.

3.4 Correlation coefficients of antioxidants and their activities

The correlation coefficients (R^2) of antioxidant compounds (including of TPC, TC,

ascorbic acid, carotene, and tocopherol) with three of antioxidant activity assays are shown in Table 4. TPC, TC, and ascorbic acid correlated strongly with %DPPH of inhibition, FRAP, and TEAC activities, indicating the important contribution of these antioxidants to antioxidant activity assays. The correlation coefficient of TPC was the highest, followed by TC and ascorbic acid, respectively. As the significant positive correlations of TPC (the average of R^2 was 0.976) and TC (the average of R^2 was 0.955) exhibited higher R^2 values between antioxidant activity assays and ascorbic acid (the average of R^2 was 0.838), these results indicated that TPC and TC exerted more antioxidant ability than ascorbic acid in the *C. siamea* leaves extracts. Antioxidant activity (DPPH radical scavenging) of some medicinal plants have been reported to have strongly correlated with phenolic substances (Matsuda et al., 2001; Miliauskas et al., 2004; Katalinic et al., 2006; Maisuthisakul et al., 2007). Zhang & Lin (2008) also reported that tannins extracted from different parts of plant, such as leaves, stem bark, and twigs, showed good relationships with FRAP and DPPH radical scavenging activity. Similarly, Shahkoomahally & Ramezanian (2013) found that high antioxidant activity in persimmon were caused by the positive relationship between TPC (R^2 = 0.944) and ascorbic acid (R^2 = 0.915). In another study, it had been reported that TPC and ascorbic acid were responsible for the antioxidant activity alone or in combination (Kulkarni & Aradhya, 2005; Khanizadeh et al., 2008; Tsao et al., 2003). Based on the results of the above in this study, it can be concluded that *C. siamea* leaves extracts are a promising group of bioactive compounds (e.g. TPC, TC, and ascorbic acid contents). Besides, the antioxidant activity was not limited only to TPC, but also came from the presence of other antioxidants, such as tannins and ascorbic acid. These groups of antioxidant compositions had strong correlation with antioxidant activities, suggesting that they had been probably responsible for the free radical scavenging activity in *C. siamea* leaves.

4. Conclusions

Extraction of bioactive compounds from *C. siamea* leaves using various solvent extractions showed differences in the amount of antioxidant compounds and antioxidant activities. The results showed that methanol extracts presented the highest efficiency values for TPC, tannin contents, DPPH, FRAP, and TEAC, followed by extracts of water, ethanol, and ethyl acetate, respectively. Water extract had the highest concentration of ascorbic acid (6.59-9.43 mg of ascorbic/g dry weight). Meanwhile, ethyl acetate extract showed the highest *β*-carotene contents (16.51-28.20 mg of *β*-carotene/g dry weight) and α-tocopherol contents (0.92-1.12 mg of α-tocopherol/g dry weight). In addition, the extracts from location 3 showed the highest antioxidants and antioxidants activities, followed by locations 2, 1, and 4, respectively. The results of this research proved that the *C. siamea* leaves contained high amounts of antioxidant compounds and antioxidant activities. Moreover, the results exhibited that plants with high contents of antioxidant compositions, such as phenolic substances, tannins, and ascorbic acid, also had high capacity of antioxidant activities.

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COMPARATIVE ANALYSIS OF THE PHYSICOCHEMICAL AND ACCEPTABILITY OF ENRICHED *GARI* (FERMENTED CASSAVA PRODUCT)

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ABSTRACT

Different materials and levels have been suggested for the enrichment of staple foods like *gari* (a fermented and roasted cassava granule). The study was aimed at using the same method of production and evaluation of the physicochemical and acceptability of enriched *gari*, with soybean (90:10), melon (95:5), okra seed (95:5) and moringa leaf powder (99:1). A significant increase in crude protein content was recorded for all the enriched *gari* samples with *soy-gari* having the highest value (7.22%) and 100% *gari* the lowest value (1.52%). There was also a significant difference on the fat content with *melon-gari* having the highest value of 10.74% while 100% *gari* sample still had the lowest value of 6.34%. Similar variations and significant difference were also observed for the carbohydrate, moisture, ash and fibre contents. The enrichment materials significantly influenced the pasting characteristics of the samples in which peak, trough and back viscosities varied between 1016.50 to 2374.54, 926.00 to 1862.50 and 1666.00 to 2924.00 RVU respectively. The enriched *gari* samples also exhibited high setback and breakdown viscosity values indicating that their pastes will have lower stability against retrogradation than 100% *gari* sample. A slight difference in hydrogen cyanide, titrable acidity, swelling and water holding capacity contents were also recorded. Sensory evaluation of the *gari* showed that the 100% *gari* was most preferred for colour, taste and odour, although, melon-*gari* emerged the best in overall acceptability. Enrichment of *gari* using these food materials is therefore recommended.

1. Introduction

Cassava (*Manihot esculenta Crantz)* is a staple food for over 500 million people in tropical Africa with Nigeria being the largest producer in Africa and more than 50% of the total world production (Adebayo *et al.*, 2003, CBN, 2003). Traditional cassava products in Nigeria are *gari, lafun, fufu*, tapioca, starch, etc. The production have significantly improved with Nigeria as the largest producer (Mroso, 2003) and *gari* being the most consumed and traded of all the food products from cassava roots in Nigeria (Westby and Twiddy, 1992, Karim *et al.*, 2014). *Gari* is fermented and partially gelatinized roasted, free flowing granules (Sanni *et al.*, 2008). Its ability to store well and its acceptance as a "convenience food" are responsible for its increasing popularity in the urban areas of west and central Africa (FAO, 1998).

Reports indicate that the physiochemical properties of *gari* are moisture content between

10.3% to 12.4%, ash content ranging from 0.69% and 0.78%, fat content between 0.33% and 0.44%, crude fibre content of 0.48% to 0.66%, cyanide content of between 0.007% and 0.011%, total titratable acidity, calculated as lactic acid ranged from 0.03 and 0.04 for all samples while the pH values ranged from 4.3 and 4.5. (Sanni *et al.*, 2008, Makanjuola *et al.*, 2012, Karim *et al.*, 2014). *Gari* is low in protein, deficient in essential amino acids and therefore, have poor qualitative and quantitative protein content (Obatolu and Osho, 1992, Osho, 2003; Oluwamukomi *et al.*, 2005). There is therefore, a need to enrich *gari* with good quality protein sources that are readily available (Oluwamukomi, 2007). Several attempts have been made by enriching with soybean, (Osho, 2003; Oluwamukomi *et al.*, 2005), okra seed, (Oyelade *et al.*, 2003, Aminigo and Akingbala, 2004), melon seed (Fokou *et al*., 2004) and Karim *et al*., (2012), suggested the use of *Moringa oleifera.*

Soybean (*Glysine max*) is a legume, native to East Asia that is grown for oil and protein around the world (Alex, 2007). It is a rich pulse containing a high percentage of protein of good quality (Sanni and Sobaniwa, 1994, Kolapo and Sanni, 2005), fat and a reasonable amount of carbohydrate. It is a good source of some essential amino acids such as lysine, tryptophan and threonine. It could be consumed as a whole food or added to other food stuff to achieve a balanced meal (Alex, 2007). Results of previous studies on fortification of cassava products using soybean has shown that fortification improves nutritional quality of resulting meals Oke, 1972, Oshodi, 1985; Osho, 2003, Oluwamukomi *et al.*, 2005). Banjo and Ikenebomeh, (1996) reported an observed increase in the protein content and reduction in the swelling indices against the control sample of soybean fortified *gari*. According to Jimoh and Olatidoye, (2009) amala fortified with 10% soy flour produced not only a nutritionally balanced meal but was more stable against retrogradation and was generally more acceptable when compared to other fortification ratios.

Okra (*Abelmoschus esculentus* L.) is widely consumed as a fresh vegetable. The mature seed is known to have superior nutritional quality (Oyelade *et al.,* 2003; Aminigo and Akingbala, 2004). In an earlier study, Karakoltsides and Constantimides (1975) found that the Protein Efficiency Ratio (PER) of okra seed flour heated at 130°C for 3hrs was not different from the non-heated flour, indicating the absence of anti-nutritional factors. According to these authors, the amino acid composition of okra seed protein is similar to that of soybean and the PER is higher than that of soybean. Okra seed is known to be rich in high quality protein especially with regard to its essential amino acids relative to other plant protein sources (Oyelade *et al.*, 2003). Amingo and Akingbala, (2004) reported that okra seed-fortified ogi at 10 and 20% substitution levels were generally acceptable; okra seed is therefore a potential source of protein for the fortification of poor protein cassava products like *gari*.

Melon is a cucurbit crop that belongs to the *Cucurbitaceae* family with fibrous and shallow root system. Melon seed kernels are major soup ingredients and they are used as a thickener and flavor component of soups. Melon seeds are less expensive and widely distributed. They can contribute substantially towards obtaining a balanced diet (Fokou *et al*., 2004). They are generally a rich source of oil. Oil seeds are generally processed to yield condiments such as ‘ogiri’. According to Badejo (2010) the substitution of wheat flour with defatted melon seed flour at 5% level did not indicate any significant difference in consumer acceptability on the other hand it beefed up the protein content by about 46%.

Moringa (*Moringa oleifera Lam*) tree is considered as one of the world’s most nutritious crops and commonly found in most parts of sub-Saharan Africa (Borlaug *et al.*, 2006). Moringa leaves on dry weight basis contain up to 30% protein, 1% to 2% fat, 2.0g calcium and 30mg iron. Abiodun *et al.*, (2012) reported that the Moringa leaf has certain health benefits such as improving blood sugar

level, blood pressure and prevention of certain diseases and is also suitable for consumption by children as well as the aged. It is expected that fortification of *gari* with *Moringa oleifera* will not only improve the protein content but also result in an increase in other micronutrients such as calcium and iron. According to Karim *et al*., (2012), *amala* fortified with moringa leaf powder at 2.5% fortification level resulted in a stable product with improved nutritional quality that was generally more acceptable. This was adopted for moringa-*gari*, however the limitation in the use of moringa at 2.5% substitution level resulted in a product with poor appearance which could result in poor acceptability therefore moringa fortification ratio was reduced to 1% fortification level for the purpose of this research.

Despite these reports, a comparative study on the impact and the optimal level of these suggested materials (soybean, melon seed, okra seed and *Moringa oleifera* leaf powder) is not available. The study therefore aimed at comparative of the physicochemical and acceptability of *gari* enriched with soybean, melon seed, okra seed and *Moringa oleifera* leaf powder.

2. Materials and methods

2.1. Sources of raw materials

Freshly harvested cassava roots and *Moringa oleifera* leaf powder were obtained from the teaching and research farm of the University of Ilorin, Ilorin, Nigeria. Soybean, melon and okra were purchased from Oja-Oba Market in Ilorin, Kwara State, Nigeria. They were sorted, packed and kept until used.

2.2. Production of Enriched *Gari* Products

The soybean seeds were cleaned and sorted before steam heating for 30-45 min at 100℃ and de-hulled after cooling by rubbing between palms to remove the seed coat by floatation. The de-hulled seeds were air dried at oven temperature of 70-80℃ until they were completely dried and then dry milled into powder using magnetic Blender (SHB- 515 model by Sorex Company Limited) to obtain the soy-flour. The melon and okra seeds were sorted, cleaned and toasted in a steel pan a temperature of 80-90℃. The toasted seeds were cooled, and milled into powder to obtain the melon and okra flour. *Moringa oleifera* leaf powder was prepared according to Karim *et al.*, (2012) method. The traditional processing method of *gari* production as described by Odunfa (1998) and Akingbala *et al*., (2005) was adopted (Fig.1). The enrichment materials (soybean, melon, okra seed and *Moringa oleifera*) were added after sieving of fermented cassava roots prior to roasting to ensure uniformity in treatment.

2.3. Chemical Analysis

Proximate analysis for moisture, protein (N x 6.25), fat, ash, and crude fibre of samples were determined according to AOAC (2000) procedures. Carbohydrate contents were calculated by difference. Titritable acidity (TTA) and pH were also determined according to AOAC (2000). Hydrogen Cyanide (HCN) was determined according to the method described by Cooke (1978)

2.4. Functional Properties

2.4.1 Determination of Water Holding Capacity (WHC)

The WHC was determined by weighing 1.0 g of each *gari* sample and mixed with 10ml of water. It was then shake in Gallenkamp shaker for thirty seconds. The sample was allowed to stand at room temperature for thirty minutes, and then centrifuged at 3,500 rpm for 30min a SORVALL GLC-1 centrifuge (Model 06470, USA). The clear supernatant was discarded and the centrifuge tube was weighed with the sediment. The amount of water absorbed by the sample was determined by difference and expressed in percentage.

2.4.2. Determination of Swelling Index

The swelling index was measured using the method of Ukpabi and Ndimele (1990). Fifty grams of each *gari* samples were put into a five hundred (500) ml measuring cylinders. Three hundred ml of water was added and allowed to stand for 4hrs before observing the level of swelling. The swelling index was then calculated as the multiple of the original volume.

2.5. Pasting Properties

Pasting characteristics were determined with a Rapid Viscometer Analyzer (RVA) (Model RVA 3D+, Newport Scientific Australia). The sample of 2.5g was weighed into a dried empty canister and mixed with 25ml of distilled water as the canister was well fitted into the RVA. The slurry was heated from 50^0C to 95^0C and cooled back to 50℃ within 12 min holding time rotating the can at a speed of 160 r/min with continuous stirring of the content with a plastic paddle. The rate of heating and cooling were at a constant rate of 11.25^0C per min. Pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV), or trough viscosity (TV), final viscosity (FV), and peak time (PTime) were read from the pasting profile with the aid of thermocline for windows software connected to a computer (Newport Scientific, 1998). Breakdown viscosity (BV) was calculated as the difference between PV and MV, while total setback viscosity (TSV) was determined as the FV minus MV. All determinations were performed in triplicate.

2.6.Sensory Evaluation/Consumer Acceptability

The sensory evaluation and consumer acceptability tests were carried out using a multiple comparison test. Twenty panelists who are familiar with *gari* were selected from Faculty of Agriculture**,** University of Ilorin (both sexes, 22 to 45 years old). The products were evaluated for their sensory qualities (taste, colour,odour and overall acceptability). The panelists were made to wash their mouth with water after evaluating each product.

2.7. Statistical data analysis

All analyses with mean and standard deviations were determined in duplicates. Data were analyzed using the Analysis of Variance (ANOVA) statistical method (Statistical Analysis System version 9.2 program, SAS Inc., (2012), USA.). Means were separated using Duncan's multiple range test. Significant differences were established at $p \leq 0.05$.

3. Results and discussions

3.1. Chemical Composition of Enriched *Gari* Products

The result of proximate quality of enriched *gari* products is presented in Table I. Moisture content ranged between 8.14% and 11.96%. Soy-*gari* had the least value of 8.14% and moringa-*gari* recorded the highest value of 11.96%. The values compared favourably with the data reported by some researchers on *gari* production (Akingbala *et al.*, 2005). The optimum moisture content of *gari* has been recommended to be between 9% and 12% (Hahn, 1989). This implies that the moisture content of the enriched *gari* products fell within the recommended range and may therefore have shelf-life of over 6 months has reported for 100% *gari* ((Hahn, 1989, Akingbala *et al.*, 2005).The inclusion of the materials significantly influenced the protein content at ($p \leq 0.05$) with soy-*gari* recording the highest value (7.22%) and 100%-*gari* had the least value (1.52%). The increase in protein content may be attributed to the complementary quantity of proteins in the materials most especially the soybean. Similar results were reported on yam flour fortified with soybean (Adetuyi and Adelabu, 2011), on 'amala' fortified with moringa (Karim *et al.*, 2012) and on plantain flour enriched with okra seed flour (Jimoh *et al.*, 2009), and the findings of Aminigo and Akingbala (2004) on *ogi* fortified with okra seed flour. Similar trends were obtained for the crude fibre content which

increased from 1.73% for 100%-*gari* to 2.04% for okra-*gari*. The high crude fibre content of okra-*gari* is similar to the report of Adetuyi *et al.*, (2011) on plantain flour enriched with okra seed flour and Aminigo and Akingbala (2004) on *ogi* fortified with okra seed flour. The carbohydrate content also varied significantly ($p \leq 0.05$) between 71.02% and 78.74%. The *gari* from 100% cassava root still recorded the highest value, while the addition of the materials influenced the value of the other products. The decrease in the carbohydrate level is expected to boost the nutritional value of the products. This corroborates with the findings of some researchers on indigenous foods fortification (Aminigo and Akingbala 2004; Oluwamukomi *et al.*, 2007; Jimoh *et al.*, Adetuyi and Adelabu, 2011; Karim *et al.*, 2012).

The titratable acidity of enriched *gari* products varied significantly at ($p \leq 0.05$) between 0.13 and 0.25. The increase in titratable acidity of the enriched *gari* products may be attributed to the increase in amino acid content of the products from the fortifying materials. The result is line with the report of Oluwamukomi *et al.*, (2007) on *gari* semolina fortified with full fat soy-melon blends. The pH value of *gari* obtained from 100% cassava roots recorded the highest value of 5.9 and soy-*gari* the lowest value of 5.3. Enrichment of *gari* also influenced the hydrogen cyanide. It was observed that the hydrogen cyanide decreased with increase in the quantity of fortifying material. The hydrogen cyanide values are within the permitted level of 10mgHCN/kg of *gari* as stated by Adindu *et al.*, (2003). The level of hydrogen cyanide is very important in describing the quality and acceptability of *gari*.

3.2. Pasting Properties

The results of the pasting characteristics are shown in Table 2. The pasting temperature of the *gari* was generally lower than the boiling temperature. The pasting temperature is a measure of the minimum temperature required to cook a given food, it can have implications on the stability of other components (Newport Scientific, 1998). Hence the *gari* could form a paste in hot water. The result shows that the peak viscosity which is the maximum viscosity varied significantly at ($p \leq 0.05$) between 1016.5 and 2374.5RVU. The highest value of 2374.5RVU was recorded for okra-*gari* and soy-*gari* had the lowest value of 1016.5RVU. Okra-*gari* recorded the highest trough value of 1862.5 RVU and soy-*gari* had the lowest value of 926 RVU**.** Oguntunde (1987) reported that the associative bonding of the amylose fraction is responsible for the structure and pasting behaviour of starch granule. Peak viscosity has been reported to be closely associated with the degree of starch damage and high starch damage results in high peak viscosity (Sanni *et al*., 2001). This could be inferred as an indication of the ability of the starch granules to swell or gelatinize in hot water. The break down viscosity ranges from 72 and 512RVU with okra-*gari* having the highest value of 512RVU and moringa-*gari* gave the lowest value of 72 RVU. Adebowale *et al.*, (2005) reported that the higher the break down viscosity, the lower the ability of the sample to withstand heating and shear stress during cooking. The final viscosity also varied significantly at ($p \leq 0.05$) with values between 1666 and 3018.5 RVU. According to Shimelis *et al*., (2006) final viscosity indicates the ability of starch to form various paste or gel after cooling. The 100% *gari* had the highest final viscosity compared to the enriched *gari* products due to the replacement of starch granule responsible for this behavior with protein food materials. The variation in the final viscosity might be due to the simple kinetic effect of cooling on viscosity and the re-association of starch molecules in the samples. The set-back viscosity revealed values between 740 and 1378.50 RVU with moringa-*gari* having the highest value of 1378.50 RVU and soy-*gari* revealed the lowest value of 740 RVU. Sanni *et al.*, (2001) reported that lower set back viscosity during the cooling of *gari* indicates higher resistance to retrogradation.

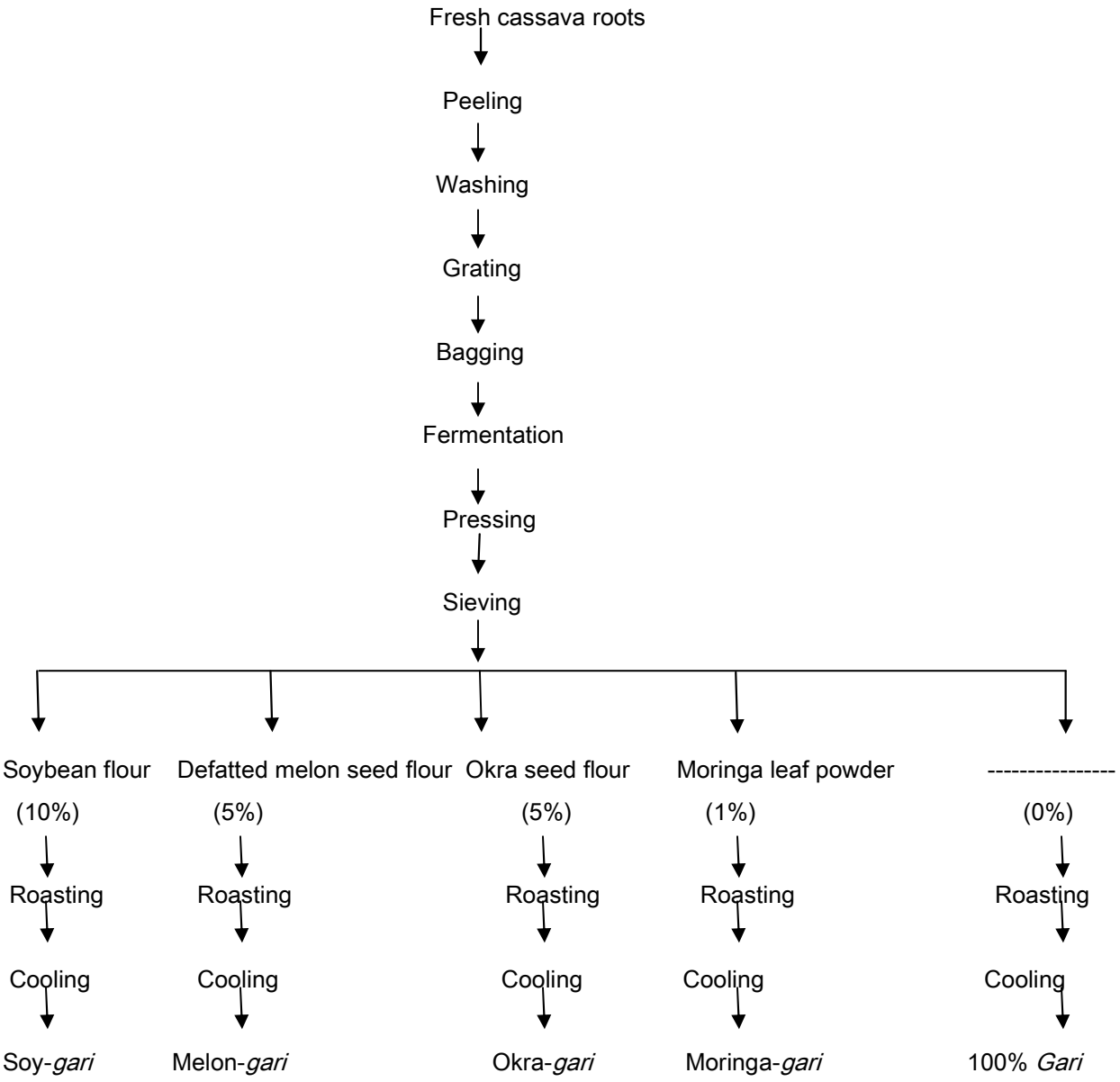

Figure 1. Flow chart for production of enriched *gari* products.

Table 1. Proximate Composition of Enriched *Gari* Products

Products	**Proximate Content (%)**					
	Moisture	**Protein**	**Fat**	**Crude Fibre**	**Ash**	**Carbohydrate**
100% *gari*	9.12^{c}±0.017	1.52^{e}±0.055	1.34^{d}±0.299	1.73^{c}±0.042	1.55c±0.030	84.74^{a}±0.242
Soy-*gari* (10%)	8.14^{e}±0.040	7.22^{a}±0.045	3.81^{b}±0.364	1.46^{d}±0.038	2.25^{ab}±0.021	77.02^{d}±0.512
Melon-*gari* (5%)	8.28^{d}±0.030	4.16^{b}±0.040	5.74^{a}±0.191	2.11^{e}±0.021	2.47^{a}±0.612	77.23^{c}±0.499
Okra-*gari* (5%)	10.26^{a}±0.026	3.54^{c}±0.118	2.61^{c}±0.332	2.04^{a}±0.079	1.76^{bc}±0.032	79.79^{c}±0.285
Moringa-*gari*(1%)	11.96^{b}±0.072	1.97^{d}±0.080	1.54^{d}±0.238	1.88^{b}±0.036	1.55^{c}±0.148	81.80^{b}±0.156

*Values represent mean of triplicates. Values with the same letter along the column are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test

Table 2. Hydrogen Cyanide, pH and Titratable Acidity of Enriched *Gari* products

***Gari* products**	**Hydrogen cyanide mg/Kg**	**pH**	**Titratable acidity %**
100%-*gari*	6.73±0.05 [a]	5.9±0.12[a]	0.13±0.02 [a]
Soy-*gari*	6.29±0.04 [a]	5.3± 0.08[a]	0.25±0.02 [a]
Melon-*gari*	6.51±0.04 [a]	5.5±0.10 [a]	0.21±0.04 [a]
Okra-*gari*	6.63±0.02 [a]	5.7±0.06 [a]	0.19± 0.01[a]
Moringa-*gari*	6.68±0.05[a]	5.7±0.12 [a]	0.14±0.04 [a]

*Values represent mean of triplicates. Values with the same letter along the column are not significantly different at (P≤0.05).

Table 3. Functional Properties of Enriched *Gari* Products

***Gari* products**	**Swelling index (%)**	**Water Holding Capacity (%)**
100%-*gari*	4.82± 0.02[a]	24.34±0.05[a]
Soy-*gari*	2.43±0.05[d]	19.22±0.03[c]
Melon-*gari*	2.22±0.04[d]	22.80±0.05[b]
Okra-*gari*	2.97±0.05[c]	23.05± 0.05[a]
Moringa-*gari*	3.93±0.02 [b]	20.54±0.02[c]

Mean values with the same letter along the column are not significantly different at (P≤0.05).

Table 4. Pasting Characteristics of Enriched *Gari* Products

***Gari* Samples**	**PT (^oC)**	**PK (RVU)**	**TR (RVU)**	**BD (RVU)**	**FV (RVU)**	**SB (RVU)**	**PKT (Min.)**
100%-*gari*	79.05[a]	1902.50[b]	1789.50[ab]	113.00[b]	3018.50[a]	1229.00[ab]	6.10[bc]
Soy-*gari*	72.35[a]	1016.50[d]	926.00[c]	90.50[b]	1666.00[c]	740.00[c]	6.60[ab]
Melon-*gari*	81.97[a]	1595.00[c]	1510.50[b]	84.50[b]	2585.50[b]	1075.00[ab]	6.06[bc]
Okra-*gari*	89.97[a]	2374.50[a]	1862.50a	512.00[a]	2818.00[ab]	955.50[bc]	5.76[c]
Moringa-*gari*	92.00[a]	1617.50[c]	1545.50[ab]	72.00[b]	2924.00[ab]	1378.50[a]	6.96[a]

Mean values with the same letter along the column are not significantly different (P≤0.05)
PK-Peak viscosity TR-Trough Viscosity BD-Break Down Viscosity FV-Final Viscosity PKT-PeakTime SB-Set Back viscosity PT-Peak Temperature RVU-Rapid Viscometer Unit

Table 5. Sensory Characteristics of Enriched *Gari* Products

***Gari* products**	**Colour**	**Taste**	**Odour**	**Overall Acceptability**
100% *gari*	1.25[b]	1.80[b]	1.55[c]	1.70[b]
Soy-*gari* (5%)	1.80[b]	2.20[b]	2.30[ab]	2.00[b]
Melon-*gari* (5%)	1.30[b]	2.10[b]	1.95[bc]	1.55[b]
Okra-*gari* (5%)	3.65[a]	3.05[a]	2.80[a]	3.40[a]
Moringa-*gari* (1%)	3.45[a]	2.90[a]	2.80[a]	3.25[a]

Mean values with the same letter along the column are not significantly different at (P≤0.05)

Higher setback value is synonymous to reduced dough digestibility while lower setback during the cooling of the paste indicates lower tendency for retrogradation. This means that soy-*gari* will exhibit higher resistance to retrogradation. The peak time varied at ($p \leq 0.05$) between 5.76 and 6.96 min as shown in Table 2. Peak time is an indication of the time taken for the starch granules to gelatinize completely and form a stir mass. The pasting temperature ranged from 72.35 and 92.0^{o}C between soy-*gari* and moringa-*gari* respectively. This indicates the temperature at which starch granules in the products formed a stiff paste. Pasting temperature is related to the moisture content of product and decreases with increased moisture content. There were no appreciable differences in the peak time and pasting temperatures of both enriched and control samples. This shows that enrichment resulted in reduced peak viscosity, trough viscosity and the breakdown viscosity, while it also resulted in increased final and setback viscosities.

3.3. Functional Properties of Enriched *gari*

The result of the functional properties (swelling index and water holding capacity) of *gari* products is shown in Table 3. Swelling capacity of the *gari* products ranged from 2.22 and 4.82% with 100% *gari* having the highest value and melon-*gari* gave the lowest value. This may be due to higher amylose starch fraction in melon-*gari*. The lower swelling power value obtained in melon-*gari* than those of other *gari* products also suggests a more highly ordered arrangement in its granules that is the lower the swelling index, the more orderly the arrangement of the starch granule. Sanni *et al*. (2005) reported that the swelling index of granules reflect the extent of associative forces within the granules, therefore the higher the swelling index, the lower the associative forces between the granules. The WHC of *gari* product varied at ($P \leq 0.05$). The result of WHC of *gari* products ranged from 19.22 and 24.34%. 100% cassava roots had the highest value of 24.34% and soy-*gari* had the least value of 19.22%. The decrease in WHC may probably be due to lose of association of amylose and amylopectin in the native granules of starch and weaker associative forces maintaining the granules structure due to the fortifying materials. The increase in the protein content of the enriched *gari* products may have accounted for the slight increase in WHC. This seems plausible since proteins have been reported to contribute to WHC of food materials (Karim *et* al., 2014, Jimoh *et al.*, 2009).

3.4. Sensory Evaluation of Enriched *Gari* Products

The ratings for taste colour, odour and overall acceptability showed that the incorporation of soybean, melon, okra and moringa to *gari* had effects (Table 5). Okra-*gari* was rated the least in terms of colour due to the appearance of dark particles from the okra seed flour. While *gari* obtained from 100% cassava roots was rated the best in terms of taste, followed by melon-*gari*, soy-*gari*, moringa-*gari* and okra-*gari* rated the least. Similar trend was observed for the odour. Despite the ratings for colour, aroma and taste, melon-*gari* was rated the best in overall acceptability while the *gari* obtained from 100% cassava roots was rated second. The trend of this result explains the influence of enrichment on the nutritional quality of *gari*.

4. Conclusions

The study revealed a general increase in the physicochemical composition of enriched *gari* samples compared to the 100% *gari*. The overall acceptability indicated melon-*gari* as the best. Therefore 5% melon *gari* is recommended as the best enriched *gari* product based on the physicochemical and overall acceptability and could therefore be employed to address the nutritional inadequacy of the community. However, studies are required on the influence of these materials on storability of *gari* and other cassava products.

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EFFECT OF PROBIOTIC *LACTOBACILLUS* STRAINS ON ANTIOXIDANT ACTIVITY FROM FERMENTED GOAT MILK

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ABSTRACT
This study mainly aimed to screen the probiotic *Lactobacillus* with high antioxidant ability during the fermentation of goat milk. The strains were selected from 25 strains by the DPPH radical scavenging activity, acidity and pH. The results showed that all the strains had antioxidant ability and 4 strains were screened due to their high scavenging rate of DPPH radical taking Trolox for reference. They were *L. plantarum* L60, *L. casei* L49 and L61, *L. acidophilus* LA5. In vitro experiments, the DPPH radical scavenging rate of fermented goat milk by the 4 *lactobacillus* strains reached to 86.68%, 81.10% , 76.13% and 69.40%, respectively. The acidity and pH of them were 68 ^{o}T, 68.8 ^{o}T, 102.4 ^{o}T, 72.4 ^{o}T and 4.80, 4.43, 4.16, 4.42, respectively.

1. Introduction

Free radicals are defined as reactive molecules, irons or atoms possessing one or more unpaired electrons, which are produced by normal metabolism (Kaufmann et al., 2002). Due to the unpaired electrons, the stability of free radicals is searched through electron pairing with biological macromolecules, such as the proteins, lipids and DNA of healthy cells (Ozyurt et al., 2007). Oxidation of biomolecules could result in mutagenic changes, tissue damages and cell death (Yang *et al.*, 2000), the risk of several diseases including cancer, senile dementia, cirrhosis, cardiopathy, cataract, diabetes could be increased (Prakash et al., 2007; Li et al., 2008; Zhao *et al.*, 2011). Thus, in order to prevent a series of diseases, antioxidants were required to delay or inhibit the consumption of organism by free radical.

Milk proteins are generally regarded as a source of energy, essential amino acid and other nutritious ingredients (Sarmadi et al., 2010), the peptides from milk, which are specific protein fragments, are especially considered to have functional activities. Antioxidant activity has been reported for milk proteins (Woo et al., 2009), yoghurt (Mccue et al., 2005; Farvin et al., 2010a; 2010b), sodium caseinate and whey proteins (Sugiarto et al., 2009; Unal et al., 2013).

Probiotics are live microorganisms that confer health benefits to human with adequate amounts (FAO/WHO, 2002; Guarner *et al.*, 1998). Lactic acid bacteria (LAB), kinds of gram-positive bacteria, are widely spread in nature and universally applicable to industrial milk fermentations (Li et al., 2012). Several important biological functions have been found on some LAB strains, such as anti-ageing and antioxidant activities (Kudoh et al., 2001; Liu et al., 2005; Saide et al., 2005; Lee et al., 2010; Ayeni et al., 2011). Furthermore, the antioxidant activity of *Bifidobacteria* has been

reported both in vitro and vivo studies (Huang et al., 2011; Shen et al., 2011; Xu et al., 2011). Thus, the aim of this study was to screen strains with antioxidant activity from 25 probiotic *Lactobacillus* strains.

2. Materials and methods

2.1 Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Chemical Co.), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, MP Biomedicals Co.) was of chromatographically pure.

2.2 Microorganism and culture conditions

The 25 probiotics strains, including 6 *Lactobacillus acidophilus* strains (L4, L5, LA5, L6, L9, L89), 5 *Lactobacillus bulgaricus* strains (L10, L28, L41, L48, L59), 3 *Lactobacillus reuteri* strains (L21, L32, L33), 4 *Lactobacillus rhamnosus* strains (L20, L35, L57, LD), 3 *Lactobacillus plantarum* strains (L60, L69), 4 *Lactobacillus casei* strains (LC, L49, L61, L83), 1 *Lactobacillus paracasei* strain (L54) were obtained College of Life Science &Engineering, Shaanxi University of Science & Technology. To the acquirement of fresh cultures, the bacterium were activated in MRS broth (Hopebio, Qingdao, China) in triplicate and cultivated at 37ºC for 24h, then were added to 14% (w/w) autoclave goat milk at 42º**C** until coagulated with 5% (v/v) inoculum.

2.3 Preparation of fermented goat milk supernatant

Took 5mL fermented goat milk into each tube and the homogenates were acidified to 3.4-3.6 using 1M HCl and centrifuged at 8000×g for 15 min. Then collected supernatants and alkalified to 8.3 using 1M NaOH and centrifuged at 8000×g for 15 min. Finally, re-collected the supernatants and samples were gained. The pH was directly evaluated through a pH-meter (pHS-3C Shanghai Precision Scientific Instrument Co., Ltd, Shanghai).

2.4.Determination of the Scavenging Activity on DPPH Radical

The DPPH scavenging activity of the probiotic yoghurt samples was determined by the method of Unal et al. with a slight of modification. 8mL 0.1mM DPPH radical solution in 95% ethanol was mixed with 2mL samples and 95% ethanol (as DPPH blank), respectively. In addition, the mixture of 8mL 95% ethanol and 2mL samples were as control samples. Then, the mixture were shaken vigorously and kept at room temperature in the dark for 30min. The absorbance of each sample was measured at 517nm. 0.25mg/mL Trolox was used as a reference antioxidant. The scavenging activity was calculated using the following equation:

$$\text{Scavenging activity}(\%)=[1-(A_i - A_j)/ A_0]\times 100\% \quad (1)$$

where: A_i is the value for 2mL sample/8mL 0.1M DPPH radical solution, A_j is the value of 2mL sample/8mL 95% ethanol, A_0 is the value of 2mL 95% ethanol/8Ml 0,1M DPPH radical solution, respectively.

2.5. Determination of the acidity

The acidity was determined using neutralization titration and expressed with Thorner degrees (^oT). 5mL fermented goat milk and 10mL distilled water were taken to 100mL conical flask with addition of 2-3 drops of 1% phenolphthalein. Then titrated using 0.1M NaOH and shook constantly until the red does not fade away in 30s. Marked the dosage of NaOH and expressed with ^oT.

3. Results and discussions

3.1.Acidity, pH and the scavenging activity of *L. acidophilus.*

The acidity, pH and the scavenging activity of DPPH radical of *L. acidophilus* were shown in Figure 1. After the goat milk coagulated, the acidity of L4, L5, LA5, L9 and L89 were 67.8^oT, 64.2^oT, 72.4^oT, 67.2 ^oT and 86 ^oT, and the pH droped to 4.45, 4.9, 4.42, 4.88 and 4.24,

respectively. The acidity of goat milk fermented by L6 reached to 152.2, while the pH droped to lower than 4, which was 3.51. All the 6 *L. acidophilus* strains showed antioxidant activities. The DPPH radical scavenging rate of L4, L5, LA5, L9 and L89 were higher than 50%, which were 61.12%, 65.16%, 69.40%, 51.74%, 52.64%, respectively. The rate of L6 was 41.67%.

3.2 Acidity, pH and the scavenging activity of *L. bulgaricus.*

As Figure 2 showed, the pH of *L. bulgaricus* were all dropped to lower than 4.5, the value of L10, L28, L41, L48 were 3.94, 4.18, 4.13, 3.86, respectively, and the pH of L59 even reduced to 3.58. However, the acidity of the 5 strains were 129.2 ^{o}T, 99.8 ^{o}T, 88.4 ^{o}T, 112.2 ^{o}T, 150.8 ^{o}T, respectively.

The scavenging activities of L10, L28, L48, L59 were at around 50%, which were 48.10%, 51.34%, 51.90%, 51.64%, respectively. The value of L41 was lower than others, which showed 41.13% scavenging rate of DPPH radical.

3.3 Acidity, pH and the scavenging activity of *L. casei, L. paracasei and L. plantarum.*

The acidity, pH and scavenging rate of L. casei, L. paracasei and *L. plantarum* were shown in Figure 3. After coagulated, the acidity of 7 strains enhanced, which were 76.2 ^{o}T, 68.8 ^{o}T, 102.4 ^{o}T, 77.8 ^{o}T, 104.2 ^{o}T, 68.0 ^{o}T, 66.2 ^{o}T, respectively. While the pH of *L. casei*(LC, L49, L61, L83) and *L. paracasei*(L54) dropped lower than 4.5 and the values of *L. plantarum*(L60, L69) were 4.80 and 4.73, respectively.

Figure 3 indicated that the yoghurt fermented by L49, L61 and L60 showed better effect on DPPH radical scavenging. The rate of L49 and L61 were 81.10% and 76.13%, and the rate of L60 was extremely high which reached to 86.68%.

3.4 Acidity, pH and the scavenging activity of *L. reuteri, L. rhamnosus*

According to the results shown in Figure 4, the difference of acidity and pH could be obtained. L20 had the highest acidity which was 115.4 ^{o}T and the lowest pH 3.75 among the 7 strains, while L32 showed lowest acidity 72.6 ^{o}T and higher pH 4.61 compared with other strains.

The DPPH radical scavenging rate of 3 *L.reuteri* strains were lower than 40%, L32 had 21.90% scavenging rate of DPPH radical, which was the lowest among strains. The scavenging activities of 4 *L.rhamnosus* strains were at around 50%. As a reference antioxidant, the DPPH radical scavenging rate of Trolox reached to 97.61%, which showed extremely strong antioxidant activity.

4. Conclusions

Among the 25 probiotic Lactobacillus strains, all the strains had antioxidant ability during the fermentation of goat milk. The results indicated that *L. plantarum* L60, *L. casei* L49 and L61, *L. acidophilus* LA5 showed potent DPPH radical scavenging activity compared with other probiotic strains, which reached to 86.68%, 81.10% , 76.13% and 69.40% in vitro experiment, respectively. The acidity and pH of them were 68 ^{o}T, 68.8 ^{o}T, 102.4 ^{o}T, 72.4 ^{o}T and 4.80, 4.43, 4.16, 4.42, respectively.

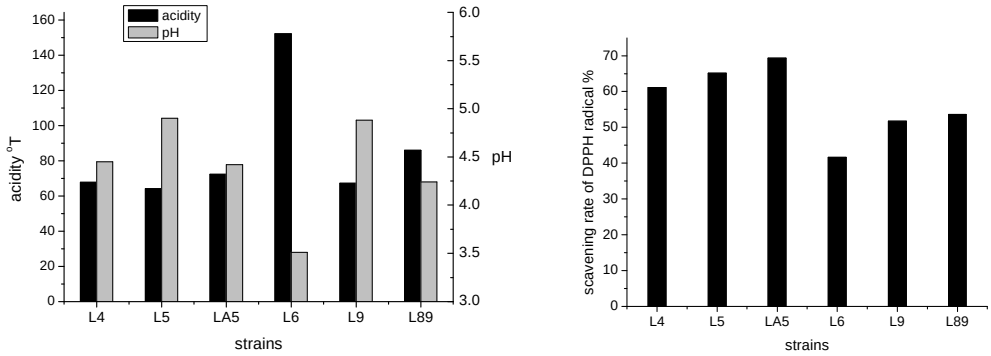

Figure 1. Acidity, pH and DPPH radical scavenging activity of *L.acidophilus*

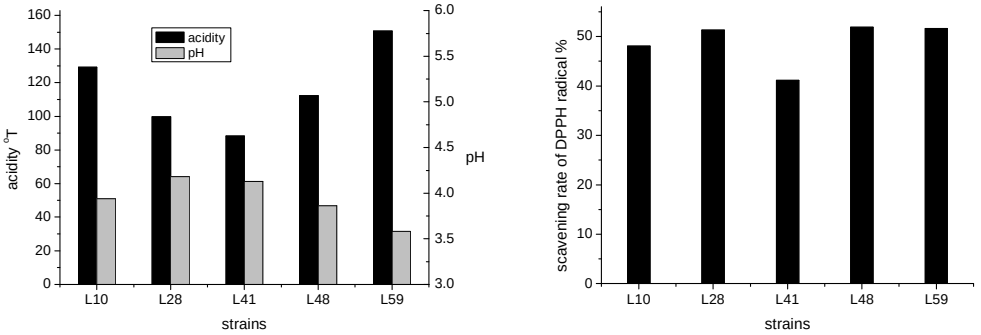

Figure 2. Acidity, pH and DPPH radical scavenging activity of *L. bulgaricus*

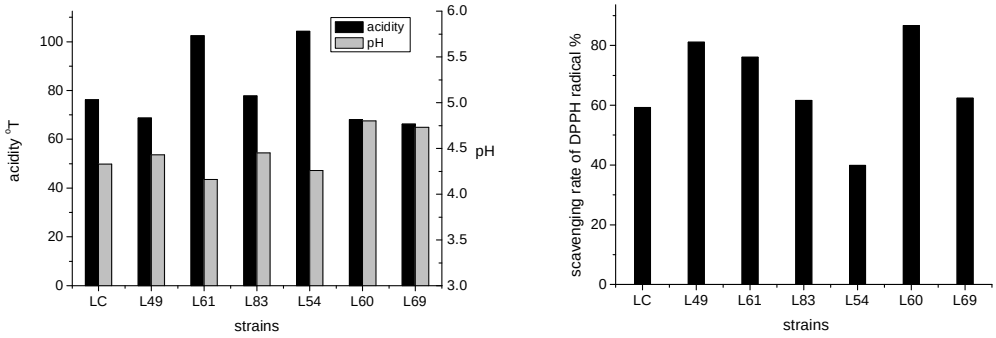

Figure 3. Acidity, pH and DPPH radical scavenging activity of *L. casei, L. paracasei and L. plantarum*

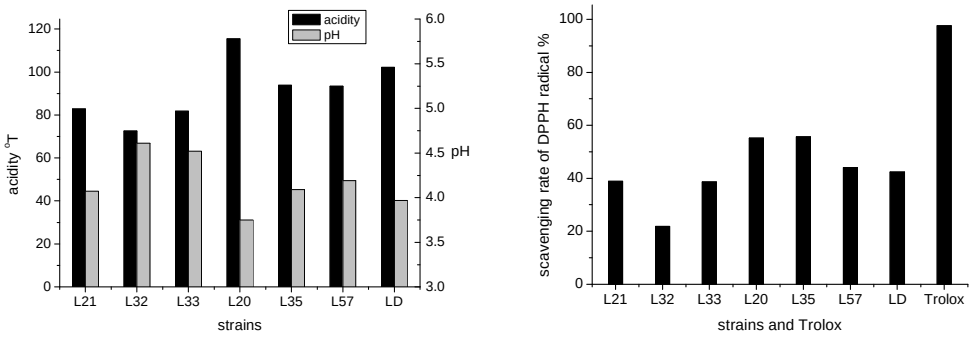

Figure 4. Acidity, pH and DPPH radical scavenging activity of *L. reuteri*, *L. rhamnosus* and Trolox

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EFFECT OF SOFTENING AGENTS ON THE CHEMICAL AND ANTI-NUTRIENT COMPOSITIONS OF FERMENTED *PROSOPIS AFRICANA* SEEDS

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ABSTRACT

The fermentation of *Prosopis africana* leads to the production of a local condiment in Nigeria called *Okpehe*. In this study, *Okpehe* was processed from *Prosopis africana* seeds using two chemical softening agents (wood ash and potash) at different concentrations (1%, 5%, 10%, 15% and 20%). Sample A was *Okpehe* processed with wood ash while potash was used for processing sample B. The effect of these softening agents on chemical and anti-nutritional (tannin, phytate and oxalate) composition of *Okpehe* was investigated every 24 hours for 72 hours. Different concentrations (1%, 5%, 10%, 15% and 20%) of the antinutrients were used for the study. There was a significant increase ($p < 0.05$) in pH (6.00 – 8.50), crude protein (32.00 – 39.50%) and crude fibre (4.10 - 6.00%) while the amounts of crude fat (12.02 – 7.20%) and carbohydrate (38.30 – 31.30%) decreased significantly ($p < 0.05$) till the end of fermentation for all the samples. There was reduction in the anti-nutritional content of *Okpehe* processed with the two softening agents as fermentation progressed. *Okpehe* processed with wood ash at 15% and that processed with potash at 20% had better chemical composition and lower anti-nutritional values. Results obtained from this study showed that wood ash is a better softening agent that can be used in the processing of *Okpehe* with an accompanying reduction in time and energy used in its production.

1. Introduction

Legumes are important sources of dietary protein in developing nations. They are traditionally available but most of their values are not well appreciated (Igwe et al., 2012). Some of these legumes like bambara groundnut, African oil bean, *Prosopis africana* seeds are underutilized. They also contain anti-nutritional factors which has contributed to their under utilization. These anti-nutrients, however, can be reduced by fermentation. Fermented plant seeds are used as food in some rural parts of Nigeria. For example *ugba* is made from African oil bean (*Pentaclethra macrophylla*) (Achi, 2005), *iru* from African locust bean (*Parkia biglobosa*), *ogiri* from castor bean (*Rucinus communis*) and *Okpehe* from *Prosopis africana* seeds (Omafuvbe et al., 2003). *Okpehe* is a traditionally fermented soup condiment produced from *Prosopis africana* seeds. The seeds are inedible in the raw form. They can only be consumed when processed and fermented using the metabolic apparatus of microorganisms. This process enhances

digestibility of proteins and oligosaccharides through hydrolysis (Oguntoyibo, et al. 2007). The processed seeds have an increased shelf life and a reduction in the anti-nutritional factors (Odunfa, 1985a). Fermentation improves digestibility, nutritive value and flavour of the raw material. *Okpehe* may serve as a substitute for meat for the low income earners (Oguntoyinbo et al. 2010), as a flavour intensifier for soups and stews and also improve the protein content of poor protein diet (Balogun et al., 2013; Oguntoyinbo et al., 2010). It also contribute to the intake of essential fatty acids (Ezenwa and Ikenebomeh, 2008), vitamins particularly B vitamins, riboflavin and vitamin A.

Prosopis africana seeds like other legumes contain anti-nutrients which are concentrated in the seed coat and dehulling becomes a critical operation in their processing. The seeds are difficult to dehull and can thus be classified as a hard to dehull legume. Several methods have been reported for the softening of the seeds. Traditionally, *Okpehe* seeds are boiled overnight to soften the seeds. Achi and Okereke (1999) reported the use of 0.1M Na_2CO_3 to boil *Prosopis africana* seeds which reduces the normal cooking time of about 5-6 hours and increased the dehulling efficiency to 89%. The addition of potash (potassium carbonate) in the softening of African oil bean seed has also been reported (Odunfa 1985b). Literature abounds on the processing of *Prosopis africana* seeds into fermented product using different methods; however, there is little or no information on the effect of softeners on the chemical composition of the fermented seeds. This study therefore, was designed to determine the effect of softening agents (wood ash and potassium carbonate) on the chemical and anti-nutrients composition of fermented *Prosopis africana* seeds.

2. Materials and methods

2.1. Vegetal samples

Prosopis africana fruits were picked from the main campus of the University of Ilorin, Ilorin, Nigeria and *Okpehe* was produced in the Department of Home Economics and Food Science Laboratory using the hot plate method described by Balogun and Oyeyiola, (2011). Ash was produced in the Department of Home Economics and Food Science Laboratory while food grade potash was obtained from a chemical score in Ilorin, Nigeria.

2.2.Preparation of *Okpehe* using hot plate

The hot plate method described by Balogun and Oyeyiola, (2011) was used with some modifications. *Prosopis africana* seeds were removed by beating the fruits with a club on a concrete surface to break the tough fruit coats. 150g of the seeds were heated at 60^{o}C for 2 hours 15mins using wood ash and potash at varying concentrations (1%, 5%, 10%, 15% and 20%) separately. The seeds of the samples were allowed to cool and the seed coats were removed by rubbing soften seeds between palms. The seed coats were washed with distilled water and decanted along, leaving the clean cotyledons. Clean cotyledons were rinsed with sterile water and cooked on the hotplate set at 60^{o}C for 30 minutes. Cooked seeds were drained with a sterile sieve and inoculated with starter cultures of *Bacillus subtilis* and *Bacillus licheniformis* (1:1) (Balogun et al., 2014). The resulting mash after inoculation was first wrapped with pawpaw leaves and then in three layers of sterile aluminium foil. The pawpaw leaves used had been cleaned and surface sterilized with 70% alcohol and rinsed with sterile water prior to their usage. The wrapped cotyledons were left at 30 ± 2^{0}C for 72 hours in an incubating unit to ferment. Wrapping time was regarded as zero hour and subsequently after every 24hours till the end of fermentation, samples were taken for analysis.

2.3.Chemical Composition Determination

Standard methods of Association of Official Analytical Chemists (AOAC, 2000) were used to determine the moisture content, crude protein, crude fat, crude fibre, ash, carbohydrate and pH of the samples, while anti-nutrient composition

of the samples was done using the method described by Amadi et al. (2004).

2.4.Statistical Analysis

All analysis except otherwise stated were in triplicates. Data generated were subjected to analysis of variance (ANOVA) and the means separated by Duncan multiple range test using statistical package for social science (SPSS) software (Version 17).

3. Results and discussions

The chemical composition of *Okpehe* fermented at 0, 24, 48 and 72 h as affected by the softening agents are presented in Tables 1, 2, 3 and 4 respectively. Generally, the chemical composition of *Okpehe* varied with softening agent and fermentation period. There were significant differences ($p \leq 0.05$) in the proximate compositions and pH values of *Okpehe* produced from seeds softened with wood ash and potash at all concentrations. At 0 h, a range of 7.58 - 11.00%, 32.00- 32.70%, 9.60-12.02%, 3.00-4.86%, 3.16-5.20%, 38.30-39.40% and 6.00-6.40 was observed for moisture, crude protein, crude fat, ash, crude fibre, carbohydrate and pH respectively for the two softeners (Table 1). A range of 10.50 - 11.70%, 35.30 - 35.90%, 10.01-11.80%, 3.20-4.85%, 3.30-6.00%, 32.70- 35.60% and 6.20 - 6.80 was observed for moisture, crude protein, crude fat, ash, crude fibre, carbohydrate and pH respectively (Table 2). A similar pattern in the physicochemical content was observed for *Okpehe* fermented for 48 (Table 3) and 72 h (Table 4). The percentage crude protein contents of *Okpehe* produced from wood ash softened seeds were higher than those produced from potash softened seeds. However, there was a reduction in the crude protein content of *Okpehe* produced from wood ash and potash softened seeds when compared to the control (*Okpehe* produced without softening agent) throughout the period of fermentation. The reduction observed in the protein content of the samples when compared to the control may be attributed to the addition of the softening agents. However there was an increase in the protein content as fermentation progressed for all the samples. This is consistent with the work of Balogun and Oyeyiola, (2011) although the values obtained in this study were lower than those reported previously for *Okpehe* by these authors. The increase in crude protein during fermentation may probably be due to the presence of enzymes in the fermenting organisms. Carbohydrate values reduced as fermentation progressed for all the samples (Tables 1, 2, 3 and 4). Reduction in the carbohydrate content of *Okpehe* may be attributed to their utilization for metabolism by the fermenting organisms. Ogunshe *et al*., (2007), reported that *Bacillus* spp. are important sources of amylase and therefore may have been involved in the degradation and subsequent utilization of this fraction in the fermented samples.

The crude fibers increased with fermentation period but no significant changes were observed when the softener concentration was varied. The percentage increase observed may be attributed to the reduction in carbohydrate fractions which may have influenced the crude fibre content. Balogun *et al*., (2013) attributed a similar increase in fermented *okpehe* to the fact that crude fibre consist largely of cellulose and hemicelluloses, which most fermenting microorganisms cannot breakdown.

The pH values observed in this study were lower than those reported by Balogun and Oyeyiola, (2011). This may be because of the addition of the softening agents. The pH values increased from 6.00 – 6.40 at 0 h to 6.60 – 8.50 at the end of 72 h fermentation period for all the samples. The increase in pH is mainly due to ammonia production, which is characterized by the pungent smell of fermented condiments (Ogunshe et al., 2007; Balogun et al., 2014).

The anti-nutrients composition of *Okpehe* as affected by the wood ash and potash are presented in figures 1 - 4. Reductions in all the anti-nutrients of the fermented samples were observed throughout the fermentation period.

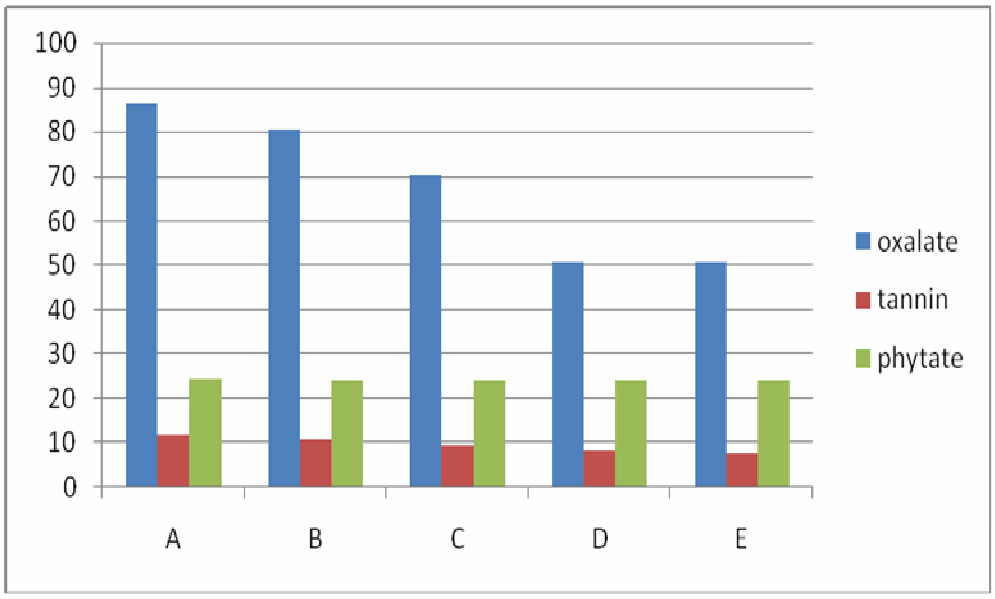

Figure 1. Anti-nutrients composition of *Okpehe p*rocessed with wood ash at various concentrations after 0 hour of fermentation
A -1%, B -5%, C -10%, D -15%, E-20%

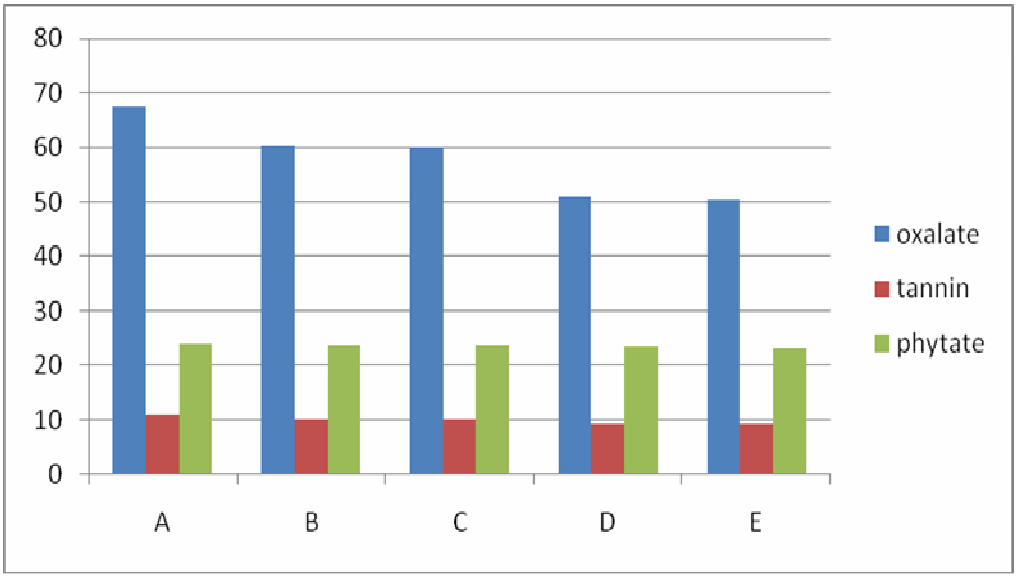

Figure 2. Anti-nutrients composition of *Okpehe p*rocessed with wood ash at various concentrations after 72 hours of fermentation

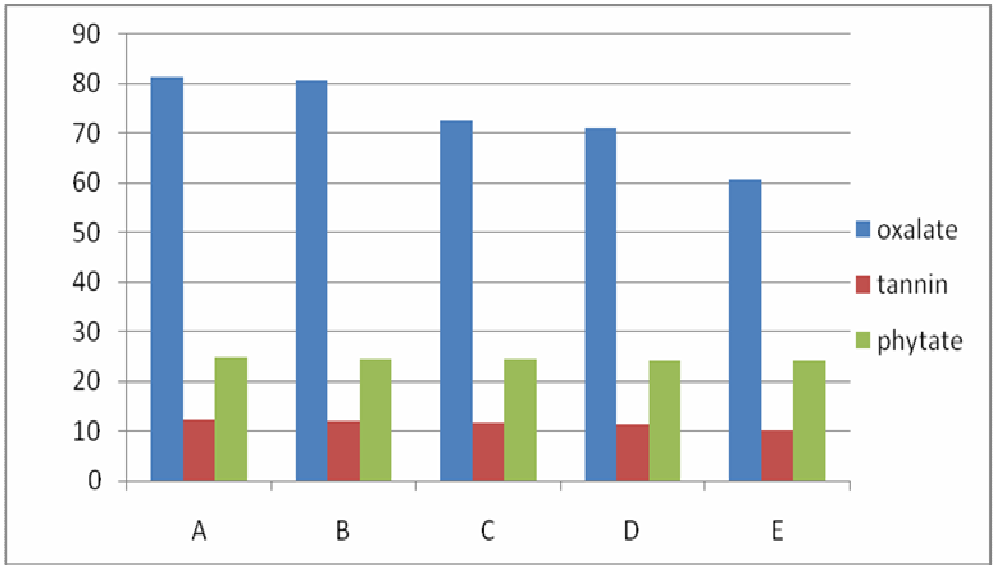

Figure 3. Anti-nutrients composition of *Okpehe* processed with potash at various Concentrations after 0 hour of fermentation

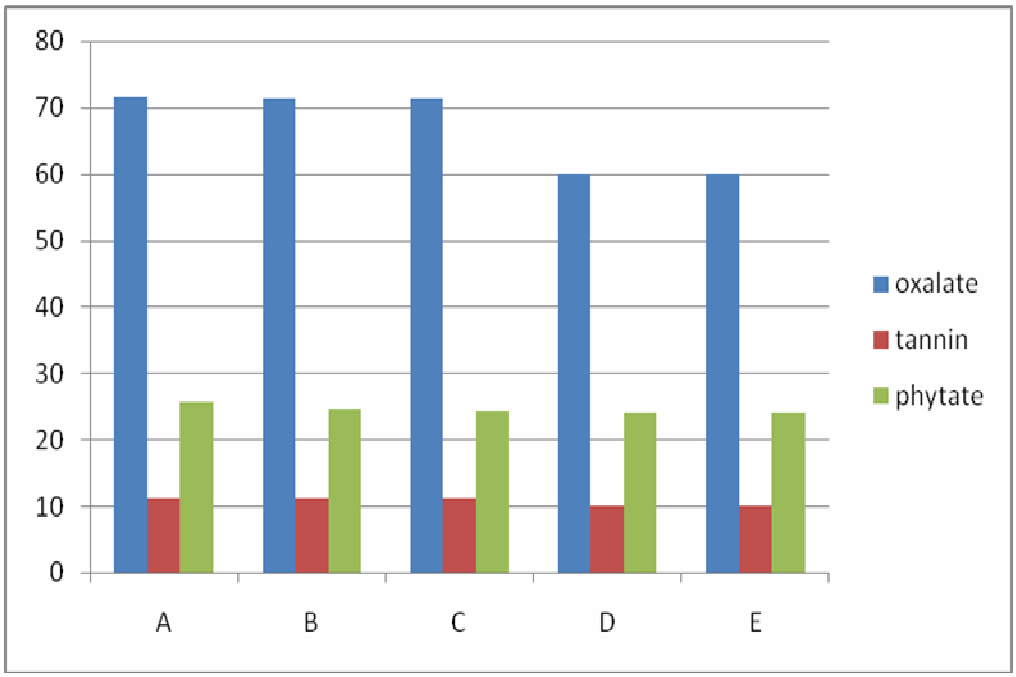

Figure 4. Anti-nutrients composition of *Okpehe* processed with potash at aarious concentration after 72 hours of fermentation

Table 1. Effect of softening agent on chemical composition of *Okpehe* at 0 hour

Concentration of softening agent (%)	Softening agent	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	CHO (%)	pH
0	-	7.58± 0.01^{e}	34.54± 0.01^{a}	11.49± 0.01^{b}	4.86± 0.01^{a}	3.16± 0.10^{c}	45.95± 0.01^{a}	6.40± 0.00^{a}
5	Wood ash	11.00 ±0.05^{a}	32.50 ± 0.02^{b}	11.60± 0.10^{b}	3.10± 0.01^{b}	4.10± 0.10^{b}	38.30 ± 0.05^{c}	6.20± 0.02^{b}
10	Wood ash	11.00 ±0.02^{a}	32.60 ± 0.02^{b}	11.80± 0.01^{a}	3.00± 0.01^{b}	4.10± 0.10^{b}	38.40 ± 0.08^{c}	6.20± 0.02^{b}
15	Wood ash	9.60± 0.02^{c}	32.70± 0.02^{b}	11.90± 0.10^{a}	3.10± 0.10^{b}	4.10± 0.10^{b}	38.60 ± 0.05^{c}	6.40 ± 0.05^{a}
20	Wood ash	8.70± 0.10^{d}	32.20 ± 0.10^{c}	12.02± 0.02^{a}	3.00 ± 0.01^{b}	4.20± 0.01^{b}	38.90 ± 0.01^{c}	6.40 ± 0.05^{a}
5	Potash	11.00± 0.01^{a}	32.00± 0.10^{c}	9.60± 0.05^{c}	3.00± 0.03^{b}	5.20± 0.01^{a}	39.20± 0.05^{b}	6.00± 0.09^{c}
10	Potash	10.80± 0.02^{a}	32.10± 0.02^{c}	9.70± 0.05^{c}	3.10± 0.10^{b}	5.00± 0.04^{a}	39.30± 0.01^{b}	6.00± 0.09^{c}
15	Potash	10.50± 0.07^{a}	32.10± 0.02^{c}	9.80± 0.02^{c}	3.20± 0.20^{b}	5.10± 0.10^{a}	39.30± 0.01^{b}	6.10± 0.10^{c}
20	Potash	10.30± 0.05^{b}	32.20± 0.05^{c}	9.90± 0.01^{c}	3.30± 0.03^{b}	5.20± 0.01^{a}	39.40± 0.01^{b}	6.10± 0.10^{c}

Values are triplicate determination; means within a column having different superscripts differ significantly ($p \leq 0.05$)

Table 2. Effect of softening agent on chemical composition of *Okpehe* at 24 hours

Concentration of softening agent (%)	Softening agent	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	CHO (%)	pH
0	-	7.83± 0.01^{a}	35.98± 0.01^{a}	11.12± 0.01^{b}	4.85± 0.00^{a}	3.30± 0.00^{d}	44.75± 0.01^{a}	6.80± 0.01^{a}
5	Wood ash	11.70± 0.02^{b}	35.70± 0.10^{b}	11.40± 0.02^{ab}	3.30± 0.05^{b}	4.20± 0.01^{c}	32.70± 0.05^{c}	6.50± 0.01^{b}
10	Wood ash	11.30± 0.02^{b}	35.80± 0.02^{b}	11.60± 0.02^{a}	3.30± 0.05^{b}	4.30± 0.05^{c}	33.70± 0.05^{c}	6.50± 0.01^{b}
15	Wood ash	11.10± 0.01^{b}	35.90± 0.04^{b}	11.50± 0.01^{a}	3.40± 0.10^{b}	4.30± 0.05^{c}	33.80± 0.01^{c}	6.50± 0.01^{b}
20	Wood ash	11.40± 0.02^{b}	35.30± 0.10^{b}	11.80± 0.10^{a}	3.30± 0.05^{b}	4.50± 0.01^{c}	33.70± 0.05^{c}	6.50± 0.01^{b}
5	Potash	11.50± 0.10^{a}	35.10± 0.01^{b}	10.01± 0.10^{b}	3.20± 0.10^{b}	5.80± 0.01^{b}	34.20± 0.10^{c}	6.20± 0.05^{c}
10	Potash	11.40± 0.09^{a}	35.30± 0.01^{b}	10.01± 0.10^{b}	3.20± 0.10^{b}	5.90± 0.10^{b}	35.10± 0.10^{c}	6.20± 0.05^{c}
15	Potash	10.60± 0.05^{b}	35.30± 0.01^{b}	10.02± 0.10^{b}	3.30± 0.05^{b}	6.00± 0.02^{a}	35.60± 0.05^{b}	6.30± 0.01^{c}
20	Potash	10.50± 0.01^{b}	35.50± 0.01^{b}	10.03± 0.10^{b}	3.20± 0.10^{b}	6.00± 0.02^{a}	35.50± 0.10^{b}	6.30± 0.01^{c}

Values are triplicate determination; means within a column having different superscripts differ significantly ($p \leq 0.05$)

Table 3. Effect of softening agent on chemical composition of *Okpehe* at 48 hours

Concentration of softening agent (%)	Softening agent	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	CHO (%)	pH
0	-	8.36± 0.01^{a}	38.01± 0.01^{a}	10.81± 0.10^{e}	4.84± 0.01^{a}	3.52± 0.00^{d}	42.82± 0.01^{b}	7.40± 0.02^{a}
5	Wood ash	12.40± 0.05^{b}	35.40± 0.10^{b}	10.40± 0.01^{d}	3.40± 0.05^{b}	5.10± 0.1^{c}	30.40± 0.05^{b}	6.60± 0.02^{c}
10	Wood ash	11.00± 0.01^{d}	36.40± 0.10^{b}	10.60± 0.01^{c}	3.30± 0.10^{b}	5.30± 0.1^{b}	30.60± 0.02^{b}	6.60± 0.02^{c}
15	Wood ash	10.70± 0.01^{e}	36.30± 0.10^{b}	10.70± 0.10^{b}	3.40± 0.03^{b}	5.40± 0.10^{b}	30.80± 0.02^{b}	6.60± 0.10^{c}
20	Wood ash	10.20± 0.1^{e}	36.40± 0.10^{b}	10.90± 0.03^{a}	3.30± 0.10^{b}	5.60± 0.10^{a}	30.90± 0.02^{a}	6.70± 0.10^{b}
5	Potash	12.40± 0.05^{b}	35.40± 0.10^{b}	10.40± 0.10^{d}	3.40± 0.05^{b}	5.10± 0.10^{c}	31.30± 0.01^{a}	6.60± 0.02^{c}
10	Potash	11.30± 0.01^{c}	36.30± 0.10^{b}	10.60± 0.10^{c}	3.30± 0.10^{b}	5.30± 0.01^{b}	31.40± 0.05^{a}	6.60± 0.02^{c}
15	Potash	11.20± 0.01^{c}	36.40± 0.10^{b}	10.70± 0.10^{b}	3.40± 0.05^{b}	5.40± 0.10^{b}	31.50± 0.10^{a}	6.60± 0.10^{c}
20	Potash	11.00± 0.01^{c}	36.40± 0.10^{b}	10.90± 0.03^{a}	3.30± 0.10^{b}	5.60± 0.10^{a}	31.60± 0.10^{a}	6.70± 0.10^{b}

Values are triplicate determination; means within a column having different superscripts differ significantly ($p \leq 0.05$)

Table 4. Effect of softening agent on chemical composition of *Okpehe* at 72 hours

Concentration of softening agent (%)	Softening agent	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	CHO (%)	pH
0	-	9.46± 0.00^{a}	40.05± 0.00^{a}	10.04± 0.00^{c}	4.84± 0.00^{a}	3.63± 0.00^{c}	41.44± 0.01^{a}	8.00± 0.00^{a}
5	Wood ash	13.50± 0.01^{c}	39.20± 0.01^{b}	10.40± 0.01^{a}	3.50± 0.10^{b}	5.60± 0.05^{b}	31.30± 0.01^{c}	7.00± 0.03^{b}
10	Wood ash	13.50± 0.01^{c}	39.20± 0.01^{b}	10.30± 0.05^{a}	3.50± 0.10^{b}	5.60± 0.05^{b}	31.40± 0.01^{c}	7.00± 0.03^{b}
15	Wood ash	13.50± 0.02^{c}	39.50± 0.10^{b}	10.40± 0.01^{a}	3.50± 0.10^{b}	5.70± 0.02^{b}	31.50± 0.10^{c}	7.20± 0.08^{b}
20	Wood ash	13.40± 0.10^{c}	39.40± 0.50^{b}	10.40± 0.01^{a}	3.60± 0.01^{b}	5.70± 0.02^{b}	31.60± 0.10^{b}	7.20± 0.08^{b}
5	Potash	15.60± 0.02^{b}	38.30± 0.10^{c}	7.20± 0.01^{b}	3.40± 0.80^{b}	5.90± 0.01^{ab}	31.90± 0.01^{b}	6.60± 0.01^{c}
10	Potash	15.60± 0.02^{b}	38.30± 0.10^{c}	7.30± 0.02^{b}	3.30± 0.10^{b}	5.90± 0.01^{ab}	31.80± 0.01^{b}	6.70± 0.01^{c}
15	Potash	15.70± 0.01^{b}	38.40± 0.01^{c}	7.30± 0.02^{b}	3.40± 0.80^{b}	6.00± 0.02^{a}	31.70± 0.10^{b}	6.80± 0.10^{c}
20	Potash	15.40± 0.01^{b}	38.60± 0.05^{c}	7.50± 0.01^{b}	3.40± 0.80^{b}	6.00± 0.02^{a}	31.80± 0.01^{b}	8.50± 0.01^{a}

Values are triplicate determination; means within a column having different superscripts differ significantly ($p \leq 0.05$)

Phytate content of the *Okpehe* however, did not change significantly at 24 and 48 h of fermentation with both softening agents. Similarly, reductions in the anti-nutrients were also observed as the concentration of the softening agent increased. The oxalate content of *Okpehe* was relatively higher than the tannin and phytate contents of all the samples. Fermentation at 72 h showed the highest reduction in the anti-nutrients composition of *Okpehe*, with both softening agents. The reduction observed in the anti-nutrients is consistent with the work of Achi, (2005), Okwu and Ndu, (2006,) and Parkouda *et al*., (2009) where it was reported that fermentation helps in the reduction of anti-nutritional factors in foods.

4. Conclusions

This study showed that the processing of *Okpehe* could be improved upon with the utilization of potash and wood ash as softeners so as to reduce time and energy usually wasted before the end product is obtained by the local producers. *Okpehe* can therefore be produced with ease and minimal loss of nutrients as shown by the proximate composition of the samples. Furthermore, the antinutrient content of the samples reduced considerably after fermentation. More investigation should however, be done on the components of these softeners so as to ensure their safety for human consumption.

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EFFECT OF ADDING SMOKE LIQUID OR POWDER TO PICKLING WHEY ON SOME PROPERTIES OF DOMIATTI CHEESE MADE FROM GOAT'S MILK

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ABSTRACT

The effect of adding smoke liquid and powder to pickling whey on the quality of Domiatti cheese was studied. Seven treatments of cheese were made from goat's milk and pickled in whey contained 0.0, 0.8, 1.0 and 1.5% smoke liquid or 1.0, 1.5 and 2.0% smoke powder. The obtained results showed that addition of smoke liquid and powder to pickling whey raised the acidity and total phenols and reduced pH values of Domiatti cheese. The total solids, fat, salt, salt in moisture and total nitrogen contents were nearly same for control and other treatments. The values of water soluble nitrogen, non-protein-nitrogen, total volatile fatty acids and saturated fatty acids were more abundant in control as compared with those of smoked cheese. Values of unsaturated fatty acids had the opposite trend. Smoked Domiatti cheese had lower contents of short chain fatty acids and slightly higher values of medium and long chain fatty acids than those of control. Pickling of Domiatti cheese in smoked whey decreased the total viable bacterial counts and the viable counts of proteolytic, lipolytic bacteria, moulds and yeasts. All smoked treatments showed higher sensory evaluation score as compared with control.

1. Introduction

Smoking of foods is one of the oldest methods of food preservation but, presently, foods are smoked for sensory quality rather than for preservative effect. In general, smoking infuses the high-protein food with aromatic components, which lend flavor and color to the food (Amran and Abbas, 2011). In the past, a natural smoke, generated by burning wood or sawdust, was used to smoke cheeses when they were placed in the vapors of smoke. Over the past 25 to 30 years liquid smoke, or natural smoke flavoring, has been used to smoke cheese (Wendorff, 2010). The liquid smoke could be applied as antibacterial, antioxidant and flavoring agents (Yusnaini et al., 2012). The most common smoked varieties of cheese are Seretpanir (Iran), Caramakase (Germany), Bandal (India), and Provolone (Italy).

Goat milk and its products are important sources of protein for humans in many developing countries. However, its production and handling presents a major problem limiting its consumption. Most goat milk cheeses are manufactured from raw goat milk with or without thermal treatment (Klinger and Rosenthal, 1997). In Egypt great numbers of citizens do not prefer it because of its aroma. Therefore, the objective of this work was to study the quality of Domiatti cheese made from goat's milk as affected by pickling in smoked whey.

2. Materials and methods

2.1. Samples

Fresh goat's milk (12.82% TS, 6.66 pH, 0.16% titratable acidity, 4.1% fat and 3.94% total protein) was obtained from El-Serw Animal Production Research Station, Animal Production Research Institute, Agriculture Research Center. Egypt. The smoke liquid was obtained from Food Technology Research Institute, Agricultural Research Center, Egypt. It was prepared according to the technique reported by Abd El-Aty (1994). Smoke powder was gotten from The French Company for Food Industries, 6th of October city, Egypt. Liquid calf rennet (single strength) obtained from local market (Damietta city) was added to the milk at a rate of 1.5 mL kg^{-1} milk. Dry coarse commercial food grad salt was obtained from El-Nasr Salines Company, Egypt.

2.2. Cheese manufacture

Domiatti cheese was made from goat's milk as described by Ismail and Hamad (2012). Seven treatments of cheese were manufactured as follow:

Treatment A: Domiatti cheese (control)

Treatment B: Domiatti cheese pickled in whey contained 0.8% smoke liquid (50%)

Treatment C: Domiatti cheese pickled in whey contained 1.0% smoke liquid (50%)

Treatment D: Domiatti cheese pickled in whey contained 1.5% smoke liquid (50%)

Treatment E: Domiatti cheese pickled in whey contained 1.0% smoke powder

Treatment F: Domiatti cheese pickled in whey contained 1.5% smoke powder

Treatment G: Domiatti cheese pickled in whey contained 2.0% smoke powder

Goat's milk was heated to 40°C then salt was added at 12% and finally milk was renneted. After complete coagulation, the resultant curds were ladled in wooden frames, lined with muslin cloth. After 24 hours, the resultant cheese of all treatments were weighed and pickled into their own whey which contained the above mentioned smoke liquid or powder amounts. The cheese samples were stored in plastic jars at 25°C for 3 months. Samples of cheese were analyzed when fresh and after 15, 30, 60 and 90 days of ripening period. Three replicates of each treatment were conducted.

2.3. Chemical analysis:

Total solids, fat and TN contents of samples were determined according to (AOAC, 2000). Titratable acidity in terms of % lactic acid was measured by titrating 10 g of sample mixed with 10 ml of boiling water against 0.1 N NaOH using phenolphthalein indicator to an end point of faint pink color (Parmar, 2003). pH of the sample was measured using a pH meter (Corning pH/ion analyzer 350, Corning, NY) after calibration with standard buffers (pH 4.0 and 7.0). Water soluble nitrogen (WSN) and non-protein-nitrogen (NPN) contents were estimated by the Kjeldahl method according to the AOAC (2000). Salt contents were estimated using Volhard method according to Richardson (1985). Salt-in-moisture content was calculated as the percentage of salt in the cheese based on combined moisture plus salt content. Total volatile fatty acids (TVFA) were determined as described by Kosikowski (1978) and expressed as ml of 0.1 N NaOH / 100 g^{-1} cheese. The method of Zheng and Wang (2001) was followed in determining the total phenols compounds cheese using Folin Ciocalteu Reagent (FCR) and gallic acid as a standard liquid. For determination of free fatty acids by gas liquid chromatography, lipids were extracted as outlined by Kates (1972). Free fatty acids were separated from the lipid extract according to the method of Borgstrom (1952). Methylation of free fatty acids was carried out according to the method described by Vogel (1975).

2.4. Microbiological analyses

Cheese samples were analyzed for total viable bacterial count (TVBC), proteolytic, lipolytic, moulds and yeast counts according to the methods described by the APHA (1992).

2.5. Sensory evaluation

A panel of 10 trained panelists (aged between 25 and 35 years) described the sensory character of the cheeses. The score points were 50 for flavour, 35 for body and texture, 15 for colour and appearance, which give a total score of 100 points.

2.6. Statistical Analysis

The obtained results were statistically analyzed using a software package (SAS, 1991) based on analysis of variance. When F-test was significant, least significant difference (LSD) was calculated according to Duncan (1955) for the comparison between means. The data presented, in the tables, are the mean (± standard deviation) of 3 experiments.

3. Results and discussions

3.1. Chemical analysis of Domiatti cheese:

The acidity of cheese in all treatments increased gradually ($P<0.001$), while pH values significantly decreased ($P<0.001$) as ripening period advanced (Table 1). Also, the same table shows fast increase in acidity during the first month of ripening followed by slight and gradual increase in titratable acidity during the rest of ripening period. This coincides with the reported changes in cheese microflora being increased during the first month of ripening and then decreased. Similar results were found by Karagozlu et al., (2009). From the foregoing results, it is clear that the addition of smoke liquid and powder to pickling whey raised the acidity and reduced pH values of repined Domiatti cheese. The acidity ratios of samples A, C and F after 60 days of repining period were 2.64, 2.71 and 2.68% respectively. Similar observations were reported by Khamis (2011). However, smoke liquid ability to increase cheese acidity was more than that of powder but the rates of acidity development within repining were lower for the former than those of the later.

The total solids, fat, F/DM, salt and salt in moisture contents were nearly same for control and other treatments at the beginning and during ripening period. TS, fat and salt contents of all samples significantly increased ($P<0.001$) as the ripening period progressed (Table 1). Ismail and Osman (2004) illustrated that Fat/ DM contents of Domiatti cheese made from goat's milk treatments significantly ($p< 0.001$) increased as ripening period progress reaching maximum values at the end of ripening period. This probably attributed to the decrease in solids-not fat content as a result of protein degradation and its partial loss in whey during ripening as advocated by Fayed (1982).

3.2. Total nitrogen and some ripening indices of Domiatti cheese

Table 2 presents the averages for total nitrogen, TN/DM, WSN, WSN/TN, NPN, NPN/TN and TVFA obtained in the chemical analysis of Domiatti cheese during repining period. Mixing of different concentrations of smoke liquid and powder with pickling whey did not affect the total nitrogen and TN/DM of Domiatti cheese made from goat's milk.

The level of proteolysis in the experimental Domiatti cheese as measured by WSN and NPN contents was diverse to that of the control cheese (Table 2). The values of WSN, WSN/TN, NPN, and NPN/TN were more abundant in control as compared with those of smoked cheese. Since part of proteolysis in soft cheeses such as Domiatti, is brought by the activity of microbial groups and the action of their enzymes, the significant differences ($P<0.001$) in proteolysis between the experimental and the control cheeses might have been attributed to the antibacterial effect of smoke agents especially liquid. This finding is in line with an earlier report by Khamis (2011) who showed that dipping of soft cheese in smoke liquid decreased NPN and NPN/TN contents and the decreasing rates were proportional with increasing time of dipping from zero to 90 min.

Table 1. Effect of adding of smoke liquid or powder to pickling whey on yield and some chemical properties of Domiatti cheese

Treatments	Storage period (days)	Acidity %	pH values	TS%	Fat %	Fat/ DM%	Salt %	Salt in moisture
A	0	0.43	6.38	40.65	15.88	39.06	7.12	10.71
	15	1.19	5.35	43.21	17.22	39.85	7.47	11.72
	30	2.20	4.45	45.16	18.35	40.63	7.89	12.57
	60	2.64	3.92	45.95	19.30	42.00	8.24	13.22
	90	2.88	3.80	47.18	20.82	44.12	8.66	14.08
B	0	0.43	6.38	40.65	15.88	39.06	7.12	10.71
	15	1.23	5.31	43.79	17.35	39.62	7.49	11.75
	30	2.22	4.40	45.25	18.86	41.76	7.92	12.63
	60	2.66	3.88	45.98	19.33	42.04	8.26	13.26
	90	2.91	3.79	47.36	20.85	44.02	8.67	14.14
C	0	0.43	6.38	40.65	15.88	39.06	7.12	10.71
	15	1.29	5.22	44.08	18.24	41.37	7.57	11.92
	30	2.26	4.30	45.38	19.05	41.97	7.97	12.73
	60	2.71	3.82	46.08	20.05	43.51	8.30	13.33
	90	2.95	3.74	47.12	21.08	44.73	8.70	14.13
D	0	0.43	6.38	40.65	15.88	39.06	7.12	10.71
	15	1.37	5.14	43.24	17.28	39.96	7.61	11.82
	30	2.31	4.23	45.75	18.18	39.73	8.02	12.87
	60	2.77	3.77	46.61	19.62	42.09	8.35	13.52
	90	3.00	3.70	47.50	20.77	43.73	8.73	14.25
E	0	0.43	6.38	40.65	15.88	39.06	7.12	10.71
	15	1.21	5.33	43.81	17.98	41.04	7.50	11.77
	30	2.20	4.40	45.36	18.84	41.53	7.92	12.65
	60	2.65	3.90	46.20	19.45	42.09	8.26	13.31
	90	2.89	3.79	47.59	20.95	44.02	8.68	14.21
F	0	0.43	6.38	40.65	15.88	39.06	7.12	10.71
	15	1.25	5.27	43.88	17.99	40.99	7.53	11.83
	30	2.22	4.33	45.48	18.86	41.46	7.96	12.74
	60	2.68	3.86	46.33	19.60	42.30	8.29	13.37
	90	2.90	3.77	47.61	21.06	44.23	8.71	14.26
G	0	0.43	6.38	40.65	15.88	39.06	7.12	10.71
	15	1.31	5.17	43.00	17.99	41.83	7.56	11.71
	30	2.27	4.27	45.55	18.85	41.38	7.98	12.78
	60	2.71	3.80	46.42	19.72	42.48	8.32	13.44
	90	2.92	3.74	47.78	21.25	44.47	8.72	14.31

Treatment A: Goat's milk Domiatti cheese (control)
Treatment B: Goat's milk Domiatti cheese pickled in whey contained 0.8% smoke liquid (50%)
Treatment C: Goat's milk Domiatti cheese pickled in whey contained 1.0% smoke liquid (50%)
Treatment D: Goat's milk Domiatti cheese pickled in whey contained 1.5% smoke liquid (50%)
Treatment E: Goat's milk Domiatti cheese pickled in whey contained 1.0% smoke powder
Treatment F: Goat's milk Domiatti cheese pickled in whey contained 1.5% smoke powder
Treatment G: Goat's milk Domiatti cheese pickled in whey contained 2.0% smoke powder

Table 2. Effect of adding of smoke liquid or powder to pickling whey on TN and some repining indices of Domiatti cheese

Treatments	Storage period (days)	TN %	TN/DM %	WSN %	WSN/TN %	NPN %	NPN/TN %	TVFA*	Total phenols (mg/100g)
A	0	2.27	5.58	0.238	10.48	0.080	3.52	9.75	-
	15	2.51	5.81	0.301	11.99	0.107	4.26	15.68	-
	30	2.77	6.13	0.368	13.28	0.148	5.34	20.18	-
	60	2.97	6.46	0.478	16.09	0.217	7.31	25.52	-
	90	3.05	6.47	0.635	20.81	0.288	9.44	30.78	27.54
B	0	2.26	5.57	0.224	9.91	0.065	2.87	9.49	-
	15	2.48	5.66	0.291	11.73	0.091	3.66	15.45	-
	30	2.74	6.06	0.358	13.06	0.131	4.78	20.04	-
	60	2.96	6.43	0.471	15.91	0.197	6.65	25.32	-
	90	3.03	6.39	0.627	20.69	0.269	8.87	30.30	38.19
C	0	2.25	5.50	0.218	9.68	0.053	2.35	9.38	-
	15	2.47	5.60	0.281	11.37	0.077	3.12	15.25	-
	30	2.75	6.06	0.347	12.61	0.119	4.32	19.88	-
	60	2.94	6.38	0.458	15.57	0.185	6.29	25.22	-
	90	3.03	6.43	0.612	20.19	0.258	8.51	30.13	47.47
D	0	2.24	5.47	0.211	9.42	0.050	2.23	9.17	-
	15	2.47	5.71	0.273	11.05	0.076	3.07	15.14	-
	30	2.72	5.94	0.339	12.46	0.117	4.30	19.65	-
	60	2.93	6.28	0.441	15.05	0.182	6.21	25.10	-
	90	3.02	6.35	0.593	19.63	0.254	8.41	29.95	57.11
E	0	2.26	5.55	0.235	10.39	0.079	3.49	9.68	-
	15	2.49	5.68	0.299	12.01	0.106	4.25	15.64	-
	30	2.76	6.08	0.368	13.33	0.141	5.11	20.11	-
	60	2.97	6.42	0.480	16.16	0.213	7.17	25.50	-
	90	3.07	6.45	0.638	20.78	0.285	9.28	30.68	34.10
F	0	2.24	5.50	0.233	10.40	0.086	3.84	9.53	-
	15	2.46	5.61	0.297	12.07	0.112	4.55	15.55	-
	30	2.73	6.00	0.364	13.33	0.146	5.34	19.95	-
	60	2.95	6.37	0.475	16.10	0.217	7.35	25.35	-
	90	3.06	6.42	0.634	20.72	0.290	9.47	30.49	42.38
G	0	2.23	5.46	0.228	10.22	0.089	3.99	9.47	-
	15	2.46	5.72	0.295	11.99	0.114	4.63	15.39	-
	30	2.72	5.97	0.360	13.23	0.152	5.58	19.78	-
	60	2.94	6.33	0.468	15.92	0.225	7.65	25.32	-
	90	3.04	6.36	0.628	20.66	0.294	9.67	30.35	51.43

*expressed as ml 0.1 NaOH 100 g^{-1} cheese

Table 3. Free fatty acids (FFA) contents (as percent of total) in Domiatti cheese after 90 days of repining

Fatty Acids	**C**	**Treatments**						
		A	**B**	**C**	**D**	**E**	**F**	**G**
Saturated fatty acids (SFA) %								
Caprylic	8:0	4.10	2.00	1.98	1.84	2.54	2.12	2.05
Capreic	10:0	7.21	6.48	6.29	6.19	6.63	6.53	6.41
Lauric	12:0	2.70	2.41	2.39	2.31	2.76	2.91	2.62
Myristic	14:0	7.52	6.64	6.73	6.46	7.48	6.69	6.33
Pentadecanoic	15:0	1.29	0.81	0.78	0.60	0.51	0.30	0.15
Palmitic	16:0	26.86	28.80	28.72	28.45	27.8	28.38	28.29
Heptadeanoic	17:0	1.21	1.37	1.33	1.40	1.28	1.34	1.30
Stearic	18:0	12.40	12.50	12.41	12.68	12.44	12.91	13.20
Arachidic	20:0	0.24	0.21	0.32	-	0.53	-	0.18
Total		63.53	61.22	60.95	59.93	61.97	61.18	60.53
Unsaturated fatty acids (USFA)%								
Tetradecenoic	14:1n5	-	0.11	0.10	0.15	0.11	0.10	0.15
Mryistoleic	14:1ω7	-	0.16	0.17	0.20	0.11	0.15	0.15
Cis-10 pentadecanoic	15:1n6	0.27	0.21	0.20	0.24	0.23	0.25	0.25
	16:1ω5	0.56	0.52	0.57	0.59	0.62	0.65	0.77
	16:1ω7	0.97	1.39	1.77	1.93	1.14	1.66	1.85
Palmitioleic	16:1ω9	0.47	0.42	-	0.42	0.49	0.50	0.45
Hexagonic	16:3ω4	0.37	0.32	0.34	0.44	0.37	0.49	0.37
6-Octadecosaenoic	18:1ω5	0.46	0.33	0.33	0.40	0.31	0.45	0.48
Vaccinic	18:1ω7	0.80	0.95	0.78	0.85	0.85	0.76	0.94
Oleic	18:1ω9	26.99	26.30	26.80	26.43	26.01	26.90	26.99
	18:2ω5	0.38	-	0.23	-	0.16	-	0.19
Linoleic	18:2ω6	3.69	4.06	4.02	4.38	4.25	4.28	4.80
Linolenic	18:3ω3	0.31	0.39	0.42	0.63	0.40	0.41	0.41
Gamma Linolenic	18:3ω6	-	0.10	0.14	-	-	0.15	-
Octadecatetraenoic	18:4ω3	0.59	0.43	0.26	0.22	0.22	0.57	0.38
	20:1ω7	-	0.11	-	-	-	0.10	-
Gadoleic	20:1n9	-	0.17	0.18	0.25	0.22	0.30	-
Arachidoneic	20:4ω6	-	0.32	0.27	0.21	0.30	0.40	0.15
Non identified fatty acid		0.62	2.49	2.52	2.75	1.97	0.70	1.14
Total		36.48	38.78	39.10	40.09	37.76	38.82	39.47

Table 4. Effect of adding of smoke solution or powder on some microbial groups of Domiatti cheese

Treatments	Storage period (days)	TVBC (x 10^6)	Proteolytic bacteria (x 10^3)	Lipolytic bacteria (x 10^3)	Moulds & Yeast (x 10^3)
A	0	423	37	21	50
	15	289	18	16	35
	30	202	13	11	24
	60	149	10	7	13
	90	94	7	5	7
B	0	408	32	18	46
	15	251	14	14	32
	30	186	9	10	21
	60	126	5	4	10
	90	79	1	2	4
C	0	400	29	16	44
	15	244	12	12	30
	30	279	7	9	19
	60	122	4	3	8
	90	67	1	1	3
D	0	391	25	13	41
	15	233	10	10	28
	30	265	6	7	17
	60	112	2	3	6
	90	57	1	1	2
E	0	419	36	20	48
	15	286	17	16	34
	30	199	12	11	22
	60	143	9	7	12
	90	92	5	3	7
F	0	415	34	19	46
	15	280	15	15	32
	30	196	11	9	21
	60	140	8	7	11
	90	88	5	4	5
G	0	410	33	18	45
	15	273	13	15	31
	30	189	11	11	20
	60	134	8	7	10
	90	86	5	3	4

Table 5. Sensory evaluation of Domiatti cheese as affected by adding of smoke liquid or powder to pickling whey

Treatments	Storage period (days)	Color & Appearance (15)	Body & Texture (35)	Flavour (50)	Total (100)
A	0	14	33	40	87
	15	14	34	40	88
	30	14	34	40	88
	60	14	34	41	89
	90	13	34	41	88
B	0	14	33	42	89
	15	14	33	43	90
	30	14	34	43	91
	60	14	34	44	92
	90	13	34	46	93
C	0	14	33	43	90
	15	14	33	43	90
	30	14	34	44	92
	60	13	34	45	92
	90	13	34	46	93
D	0	14	33	43	90
	15	14	33	43	90
	30	14	33	44	91
	60	13	34	45	92
	90	12	34	47	93
E	0	14	33	42	89
	15	14	33	43	90
	30	14	33	44	91
	60	13	33	44	90
	90	12	34	45	91
F	0	14	32	42	88
	15	14	33	43	90
	30	13	33	44	90
	60	13	33	45	91
	90	12	34	46	92
G	0	14	32	43	89
	15	14	33	43	90
	30	13	33	44	90
	60	12	33	45	90
	90	12	34	46	92

Table 6. Statistical analysis of cheese treatments

Analysis	Effect of cheese treatments							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**LSD**
Acidity	1.86^{d}	1.89^{c}	1.92^{b}	1.97^{a}	1.87^{d}	1.89^{c}	1.92^{b}	0.011***
pH	4.77^{a}	4.75^{c}	4.69^{e}	4.64^{g}	4.75^{b}	4.72^{d}	4.67^{f}	0.007***
TS	41.83^{b}	44.61^{a}	44.66^{a}	44.75^{a}	44.72^{a}	44.79^{a}	44.68^{a}	2.77
Fat	18.31^{g}	18.45^{e}	18.85^{a}	18.34^{f}	18.62^{d}	18.67^{c}	18.72^{b}	0.027***
Salt	7.87^{e}	7.89^{d}	7.93^{b}	7.96^{a}	7.89^{d}	7.91^{c}	7.94^{b}	0.014***
Salt in moisture	12.46^{e}	12.52^{d}	12.56^{c}	12.63^{a}	12.53^{d}	12.58^{bc}	12.59^{b}	0.025***
TN	2.71^{a}	3.02^{a}	2.68^{a}	2.67^{a}	2.71^{a}	2.68^{a}	2.68^{a}	0.356
WSN	0.40^{b}	0.39^{b}	0.38^{b}	0.37^{b}	0.41^{b}	0.40^{b}	0.46^{a}	0.052
NPN	0.16^{c}	0.15^{e}	0.14^{f}	0.13^{g}	0.16^{d}	0.17^{b}	0.17^{a}	0.001***
TVFA	20.38^{a}	20.12^{d}	19.97^{f}	19.80^{g}	20.32^{b}	20.17^{c}	20.06^{e}	0.013***
Total Phenol	27.54^{g}	38.19^{e}	47.47^{c}	57.11^{a}	34.1^{e}	42.38^{d}	51.43^{b}	0.026***
TVBC	231.4^{a}	210^{c}	222.4^{b}	211.6^{c}	227.9^{a}	219.8^{b}	218.4^{b}	4.562
Proteolytic bacteria	17^{a}	14^{d}	10.6^{e}	8.8^{f}	15.8^{b}	14.6^{c}	14^{c}	1.055***
Lipolytic bacteria	12^{a}	9.6^{c}	8.2^{d}	6.7^{e}	11.4^{ab}	10.8^{b}	10.8^{b}	0.772***
Moulds & Yeast	25.8^{a}	22.6^{cd}	20.8^{e}	18.8^{f}	24.6^{b}	23^{c}	22^{d}	0.896***
Color	13.8^{a}	13.8^{a}	13.6^{ab}	13.4^{ab}	13.4^{ab}	13.2^{ab}	13^{b}	0.728
Body	33.8^{a}	33.6^{ab}	33.6^{ab}	33.4^{ab}	33.2^{ab}	33^{b}	33^{b}	0.728
Flavor	40.4^{c}	43.6^{b}	44.2^{ab}	44.4^{a}	43.6^{b}	44^{ab}	44.2^{ab}	0.758
	Effect of storage time (days)							
	0	15	30	60	90	LSD		
Acidity	0.43^{e}	1.26^{d}	2.24^{c}	2.68^{b}	2.92^{a}	0.009***		
pH	6.38^{a}	5.25^{b}	4.34^{c}	3.84^{d}	3.76^{e}	0.006***		
TS	40.65^{b}	41.72^{b}	45.42^{a}	46.22^{a}	47.45^{a}	2.341		
Fat	15.88^{e}	17.72^{d}	18.69^{c}	19.58^{b}	20.97^{a}	0.023***		
Salt	7.12^{e}	7.53^{d}	7.94^{c}	8.28^{b}	8.69^{a}	0.012***		
Salt in moisture	10.71^{e}	11.78^{d}	12.71^{c}	13.36^{b}	14.19^{a}	0.022***		
TN	2.25^{b}	2.47^{b}	2.94^{a}	2.97^{a}	3.04^{a}	0.301		
WSN	0.22^{e}	0.32^{d}	0.37^{c}	0.46^{b}	0.62^{a}	0.043***		
NPN	0.07^{e}	0.09^{d}	0.13^{c}	0.20^{b}	0.27^{a}	0.001***		
TVFA	9.49^{e}	15.44^{d}	19.94^{c}	25.33^{b}	30.38^{a}	0.011***		
TVBC	409.43^{a}	265.19^{b}	213.71^{c}	132.28^{d}	80.47^{e}	3.586***		
Proteolytic bacteria	32.28^{a}	14.14^{b}	9.85^{c}	6.57^{d}	3.57^{e}	0.892***		
Lipolytic bacteria	17.85^{a}	14^{b}	9.71^{c}	5.42^{d}	2.66^{e}	0.652***		
Moulds & Yeast	45.71^{a}	31.71^{b}	20.57^{c}	10^{d}	4.57^{e}	0.757***		
Color	14^{a}	14^{a}	13.71^{ab}	13.14^{b}	12.42^{c}	0.615		
Body	32.71^{c}	33.14^{bc}	33.42^{ab}	33.57^{aab}	34^{a}	0.615		
Flavor	42.14^{d}	42.57^{d}	43.28^{c}	44.14^{b}	45.28^{a}	0.641		

Significant different at p< (*0.05, **0.01, ***0.001). For each effect the different letters in the means the multiple comparisons are different from each. Letters a is the highest means followed by b, c …..etc

As described in Table 2, smoking materials did not only affect the proteolysis, but also on the lipolysis as evaluated by TVFA. Smoked Domiatti cheese had slightly lower TVFA contents than those of control. This can be attributed to the effect of smoke liquid and powder on the lypolitic microorganisms. Total nitrogen, TN/DM, WSN, WSN/TN, NPN and NPN/TN values of various cheese treatments significantly ($P<0.001$) increased through ripening period. Parallel to the increase in the amount of above mentioned contents, TVFA values also increased.

On Domiatti cheese samples ripened for three months, analysis of total phenols was carried out. Results are given in Table 3. Because phenolic compounds in smoke materials added to pickling whey may be penetrated and moved into cheese block by osmotic effect so it is natural that the levels of these compounds increased in cheese after 90 days of repining period. This effect was more noticeable with smoke liquid comparing with powder. Total phenol contents of treatments A, D and G at the end of ripening period were 27.54, 57.11 and 51.43mg/100g respectively. El-Shabrawy et al., (2002) found that the pH value, and soluble nitrogen in total nitrogen (SN/ TN) of Ras cheese decreased as liquid smoke dipping time increased, while phenols and carbonyls content increased.

3.3. Free fatty acids content (FFA) of Domiatti cheese

Some investigators take FFA as a good indicator parameter for judging the cheese ripening (Gonzalez et al., 1990). Table 3 deal with the GLC analysis of fat extracted from the seven Domiatti cheese treatments. Acid content was expressed as a percent of total fat. Free fatty acids content was estimated in 90 days old cheese.

3.3.1. Saturated and unsaturated fatty acids:

Data given in table 3 revealed that the saturated fatty acids (SFA) values of cheese in all treatments were inversely proportional with the amount of unsaturated fatty acids (USFA). The amount of SFA was high in treatment A (control) and decreased in treatments B, C, D, E, F and G (smoked cheese). Values of USFA had the opposite trend. Similar data were obtained by Ammar et al., (2002). Decreasing of SFA and increasing USFA values in smoked Domiatti cheese obtained in our study raise the healthy benefit of this cheese because it is well known that unsaturated fatty acids are more important in human nutrition.

3.3.2. Short chain fatty acids (C_8 – C_{12}) (SCFA):

A difference in the concentrations of SCFA was noticed between the experimental and the control cheeses at 90 days of ripening (Table 3). Smoked Domiatti cheese by addition of smoke liquid or powder to its pickling whey had lower contents of SCFA than those of control. The values of SCFA for samples A, B, C, D, E, F and G were 14.01, 10.89, 10.66, 10.34, 11.95, 11.56, 11.08% respectively. These findings indicate that smoke agents decreased level of short-chain fatty acids which responsible for the goaty flavour in Domiatti cheese made from goat's milk. This may be attributed to the inhibition of lipoprotein lipase by smoke materials. Thus, addition of smoke liquid or powder to goat cheese whey might be of significant importance in reducing the strong flavour associated with goat milk cheeses. Chilliard et al., (1984) stated that free fatty acid generation and resulting characteristic flavour of goat milk products is due to the distribution of lipoprotein lipase in various components of the milk system.

Within short chain fatty acids distribution of Domiatti cheese, capreic acid had the highest average and was followed by lauric and caprilic acids. Barber et al., (1997) and Chilliard et al., (2000) mentioned that goat milk is slightly higher in caproic (C6), caprilic (C8) and capric (C10) acids and slightly lower in butyric (C 4) and palmitic (C16) acids as compared to cow

milk. This was explained by different regulation of mammary cells between goats and cows in the elongation process of fatty acids synthesized in the mammary gland by the fatty acid synthase complex (Barber et al., 1997). The high level of hydrogenation of dietary fatty acids in the rumen is the reason for the low level of polyunsaturated fatty acids in goat milk (Chilliard et al., 2000).

3.3.3.Medium chain fatty acids (C_{14} – C_{16}) (MCFA)

Repining of Domiatti cheese in smoked whey for 90 days slightly increased the contents of MCFA. The values of MCFA in treatments A, B, C, D, E, F and G were 38.31, 39.06, 39.41, 39.48, 38.86, 39.17 and 38.76% respectively. The levels of myristic acid (C14:0) and pentadecanoic acid (C15:0) were higher in control cheese than those of other treatments. On the contrary, smoked cheese possessed the highest contents of palmitic acid (C16:0). On the other side, palmitic acid was the predominant one of medium chain fatty acids followed by myristic acid in various cheese treatments.

3.3.4. Long chain fatty acids (> C_{16}) (LCFA)

Cheese samples A, B, C, D and E had nearly the same contents of LCFA. However the levels of these acids were higher in samples F and G. The values of LCFA treatments A, B, C, D, E, F and G were 47.07, 47.14, 47.46, 47.45, 46.97, 48.58 and 49.02% respectively. The amounts of stearic (C18:0) and oleic acids (C18:1) were similar in various cheese treatments while arachidic acid (C20:0) was not detected in samples D and F. Smoked cheese had the highest levels of linoleic acid (C18:2) and linolenic acid (C18:3) which is also aromatic and have great importance on nutrition physiology. In all cheese treatments, the predominant acid of long chain fatty acids was oleic acid followed by stearic acid.

3.4. Microbial profile of cheese

Table 4 illustrates the total viable bacterial counts (TVBC) and the viable counts of proteolytic, lipolytic bacteria, moulds and yeasts of Domiatti cheese pickled in whey contained different levels of smoke liquid and powder. Because of antibacterial effect of smoke materials, blending them with whey used in cheese pickling lowered the all above mentioned microorganisms. The lowering rates were more observed with smoke liquid. On the other side, the numbers of different microbial groups for all samples significantly ($P<0.001$) decreased within ripening period and also, the rates of decrease were higher in smoked treatments than those of control. These results are in agreement with those obtained by EL-Alfy et al., (1998) who found that a great decrease in microbiological flora of cheese by smoking, and also most of lactic acid bacteria were highly sensitive to smoke especially smoke liquid of Guava wood.

El-Shabrawy et al., (2002) cleared that smoked Ras cheese had lower counts of total bacteria, lactic acid bacteria, proteolytic bacteria, lipolytic bacteria, sporeforming bacteria and yeasts & moulds than unsmoked one, irrespective of dipping time. The counts of different microbial groups were also decreased as liquid smoke dipping time increased. Anggraini and Yuniningsih (2013) showed that acidic compounds in liquid smoke have antimicrobial properties. The antimicrobial properties will increase if there is an organic acid together with a phenol compound.

3.5. Changes in sensory evaluation of cheese

The scores gained from the resultant cheese of all treatments are shown in Table 5. Generally, the body, texture and flavour properties of Domiatti cheese of all samples improved significantly ($p < 0.001$) during the ripening period. Conversely, color and appearance scores decreased at the end of repining period. Poveda et al., (2000) stated that cheese ripening is a complex microbiological and enzymatic process

characterized by the production of compounds that lend the cheese certain aroma and texture characteristics.

All the panelists who participated in the sensory session detected differences between the experimental and the control cheeses. Of course, this can be attributed to the smoke liquid or powder added to whey. Those panelists also commented that Domiatti cheese pickled in whey contained smoke powder had a light brown color which decreased the color and appearance scores at the end of repining period. The cheese pickled in whey mixed with various levels of smoke materials showed body and texture scores very closed to control cheese.

It is noticed from Table 5 that most differences between various treatments were in flavour scoring. The sample D had the highest flavour scores either fresh or during ripening. Generally speaking, all smoked treatments showed higher score as compared with control. Furtherance of these results, sample D had the lowest content of short chain fatty acids (Table 3) which award goaty flavour to cheese made from goat's milk.

El-Shabrawy et al., (2002) showed that increasing liquid smoking time yielded Ras cheese having darker colour, higher smoke flavour, and overall preference scores.

4. Conclusions

According to data obtained in the current study, it can be concluded that adding of smoke liquid or powder to pickling whey improve the microbiological quality and flavour of goat milk Domiatti cheese without any detrimental effect to the chemical composition of such cheese.

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EFFECT OF OSMOTIC DEHYDRATION AND ULTRASOUND TREATMENT ON WATER LOSS AND SOLID GAIN OF WATERMELON RIND

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ABSTRACT

Studies were conducted to use ultrasound treatment in watermelon (*Citrulus lanatus*) rind as a pre-treatment prior to osmotic-drying. A comparison with the conventional heating as pretreatment prior to osmotic-drying was also carried out. Effects of treatment time (10, 20 and 30 min in ultrasound and 1, 2 and 3 min in conventional method), sugar concentration (40, 50 and 60°Brix) and immersion time (0 - 6 h) on water loss and solid gain in watermelon rind were studied. Second order regression equations describing the effects of these factors on the water loss and solid gain were developed. Results shows the maximum water loss and solid gain of 81 and 46%, respectively for the watermelon rind treated for 30 min under ultrasound and immersed in 60°Brix solution for 6 h. In conventional pretreatment the maximum water loss and solid gain in water melon rind to the tune of 73 and 59%, respectively was achieved on treating the water melon rind for 3 min and immersed in 60°Brix solution for 6 h. The combined process (ultrasound pretreatment and osmotic-drying) was optimized for the operating conditions that minimize total processing time.

1. Introduction

Watermelon (*Citrulus lanatus*) is an important food crop in tropical countries. It has been in cultivation for a long time in India, which is often considered as the secondary center of its origin. It is a common summer crop and is grown in the lower Himalayan regions to South India. Half of a watermelon fruit is edible while the other half, consisting of about 35% rind and 15% peel, goes as waste. Watermelon is a natural and rich source of the non-essential amino acid citrulline. Citrulline is used in the nitric oxide system in humans and has potential antioxidant and vasodilatation roles. Rind contains more citrulline than flesh on a dry weight basis of 24.7 and 16.7 mg/g, respectively but a little less on a fresh weight basis of 1.3 and 1.9 mg/g, respectively. Watermelon rind is higher in dietary fiber and potassium than the flesh. Total sugar content was lower in the rind and sugars were primarily fructose and glucose, compared to flesh, which had about 30% of its sugars as sucrose (Rimando and Perkins Veazie 2005). Most tropical fruits including watermelon are highly perishable, showing a short shelf life post harvest at room temperature, which implies in losses over 30% of production (Andrade *et al*., 2007). Watermelon rind has a water content of about 95% making it susceptible to deterioration. Therefore, it is necessary to reduce the moisture content and produce shelf stable products from watermelon rind.

Drying is the most common method of food preservation and is used to reduce post-harvest loss and to produce several dried fruits, which can be directly consumed or used in processed foodstuffs. Conventional air-drying is energy intensive and consequently cost intensive because it is a simultaneous heat and mass transfer process accompanied by phase change. A pre-treatment can be used to reduce the initial water content of the fruit or can be used to modify the fruit tissue structure in a way that air-drying becomes faster. Osmotic dehydration is a water removal process involving soaking of foods, mostly fruits and vegetables, in a hypertonic solution such as concentrated sugar syrup (Falade et al., 2007). It is based on the principle that when cellular materials such as fruits and vegetables are immersed in a hypertonic aqueous solution, a driving force for water removal sets up because of the higher osmotic pressure or lower water activity of the hypertonic solution (Erle and Scherbert 2001, Jokic et al., 2007). Since the membranes responsible for osmotic transport is not perfectly selective, solutes from the solution diffuse into the product, as well. Therefore, osmotic dehydration is considered as simultaneous counter current mass transfer process. In addition, a leaching of the products own solutes (sugar, organic acids, minerals, vitamins etc), also occurs, which is quantitatively negligible compared with the first two transfers, yet essential regarding the final products composition. Water loss and solid gain depends both on operating conditions and cellular tissue type, as well as on the form in which the product was pretreated. Hence, there, is a need to identify the optimum operating conditions that increase mass transfer rates without affecting quality significantly (Eren and Keymak Ertekin 2007).

Power ultrasound is a novel technology in the food industry and its use is increasing as new uses are studied. Ultrasonic waves can cause a rapid series of alternative compressions and expansions, in a similar way to a sponge when it is squeezed and released repeatedly (sponge effect). The forces involved by this mechanical mechanism can be higher than surface tension, which maintains the moisture inside the capillaries of the fruit creating microscopic channels, which may ease moisture removal. In addition, ultrasound produces cavitation, which may be helpful to remove strongly attached moisture. Deformation of porous solid materials, such as fruits, caused by ultrasonic waves is responsible for the creation of microscopic channels that reduce the diffusion boundary layer and increase the convective mass transfer in the fruit.

Keeping these advantages of ultrasound, this study has been aimed to use the ultrasound treatment as a pre-treatment prior to osmo-air-drying of watermelon. The influence of time in ultrasound on water loss and solid gain was examined. A comparison with the conventional heating as pretreatment prior to osmo-air-drying was also carried out. The combined process (ultrasound pretreatment and osmo-air-drying) was optimized for the operating conditions that minimize total processing time.

2. Materials and methods

2.1. Samples

Mature ripe watermelons obtained from the local market were used for the experiment. Watermelon was washed and the outer green peel was hand peeled and the rind was cut into 1 cm cubes. The moisture content of the fruit was determined by heating in a drying oven at 105ºC for 48 h according to the method of the Association of Official Analytical Chemists (1990). Commercial sucrose was also purchased from the local market.

2.2.Ultrasound pretreatment

High-intensity ultrasound treatments were carried out using a lab-scale ultrasound equipment (UW 2070, Bandlein, Germany) at Food Technology Lab, SRM University, Kattankulathur, India. The equipment composed of an electric generator, a transducer,

and an amplifying horn with a radiating tip. The high frequency electrical energy was fed to a transducer where the electrical energy is transformed to the ultrasound energy through the mechanical vibration of the same frequency. The generated ultrasound was amplified in the amplifying horn and emitted through the radiating tip. An experimental set of 20 watermelon rind cubes of 1 cm was immersed in distilled water and subjected to ultrasonic waves for 10, 20 or 30 min. The water to fruit ratio was maintained at 5:1 (weight basis), which was also used in the osmotic dehydration experiments. At the end of the ultrasound pre-treatment a sample of the liquid medium was taken to determine its sugar content using Abbe refractometer (Model 4, Advance research instruments, India). This procedure was carried out to quantify the amount of sugar that the fruit loses by mass transfer to the liquid medium. These pre-treatment times were chosen after the results of kinetics studies carried out beforehand. The results showed that the effects of ultrasound pre-treatment started to influence the drying process after 10 min (little effect) and after 30 min the changes inferred in the drying process became insignificant (Fabiano et al., 2008), and for the same reason ultrasound pretreatment was done for 10, 20 or 30 min.

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2.3.Conventional pretreatment

Conventional pretreatment was performed by placing the watermelon rind in boiling water for 1 min, 2 min and 3 min, respectively.

2.4.Osmotic dehydration process

Each experimental group consisting of 20 watermelon rind cubes (Ultrasound pre-treated) was immersed in the osmotic solution for 1, 2, 3, 4, 5 or 6 h. The osmotic solution used in each experiment was prepared mixing food grade sucrose with distilled water to give a concentration of 40, 50 and 60°Brix. The osmotic solution to fruit ratio was maintained at 5:1 (weight basis) as recommended by Fernandes and Rodrigues (2007). After removal from the solution, the dehydrated samples from each group were drained, blotted with absorbent paper to remove the excess solution. Weight and moisture content were measured individually. The concentration of the solution was monitored during the runs determining the osmotic solution soluble solids content (°Brix) using Abbe refractometer. Osmotic dehydration was conducted at 50°C. Experiments were also conducted by conventional heating by dipping the watermelon rind cubes in boiling water for 1 min, 2 min and 3 min. Weight and moisture content of the samples were used to calculate the response variables namely water loss and solid gain. Water loss (WL) and Solid gain (SG) were determined using the following formula suggested by Panagiotou et al.(1999):

$$WL = \frac{MoCo - MC}{Mo} x100 \quad (1)$$

$$SG = \frac{Mo(Co-1) - M(C-1)}{Mo} x100 \quad (2)$$

where: WL is water loss, %; SG is solid gain, %; Mo is the initial weight of the sample (t = 0), g; M is the weight of the sample at time t, g; Co is the initial moisture of sample (wet basis), %; C is the moisture of sample (wet basis) at time t, %.

2.5.Statistical analysis

Whole experiments were performed in triplicate and the resulting data were analyzed using a statistical analysis program AGRES. The differences between means were compared using Duncan's multiple range test with the significance of $P<0.05$.

Essential Regression computer software, which is an MS Excel Add-In (compiled Excel Macro), was used in developing the regression models and plotting the three-dimensional surface plots to study the effect of various independent variables on dependent variable by employing multiple regression technique.

3. Results and discussions

Fresh watermelon rind was subjected to the osmotic dehydration after treating with ultrasound. Watermelon rind was also subjected to conventional treatment, which were used as control. Each treatment was replicated thrice and the osmotic dehydration was conducted for 6 h. The treated watermelon rind was assed for water loss and solid gain during the treatment period and the exhibited results are discussed below in detail.

Osmotic dehydration with and without ultrasound pretreatment was carried out at 50°C. Higher temperature during osmotic dehydration increased the water loss and solids gain due to increasing diffusion coefficients and decreasing viscosity of sucrose solution. There are limitations using high temperature such as 60°C because of lower WL/SG ratio (and or lower product quality) and the viscosity of the 70% solution at 50°C temperature was so high and its water loss curve was very close to the 60% curve. So the optimum temperature and concentration were found to 50°C and 60% respectively (Khoyi and Hesari 2007). High temperature caused swelling and plasticizing of cell membrane and in that way membrane became more permeable to water coming out of the product and in the same time higher temperature reduced the viscosity of the osmotic medium which results in better water transfer characteristics on the product surface (Simal et al., 1998, Jokic et al., 2007). The analysis of the fresh fruit showed that watermelon rind had an initial moisture content of 92.5% and a soluble solids content of 5.8°Brix.

3.1.Effect of sucrose concentration and immersion time on water loss

The data on the water loss of ultrasound treated watermelon rind as affected by different treatment period and immersion period in different concentration of sugar solution are depicted in the form of the surface plots in Fig. 1. It is evident from the figure that the increase in immersion period and sugar concentration increased the water loss of ultrasound treated watermelon rind. Water loss was higher in ultrasound pretreated watermelon rind than conventionally treated watermelon rind. However, ultrasound application caused a series of rapid alternative compression and expansion, which could expel water from the fruit to the liquid medium, as, observed by Fernandes et al., 2008 a.

An initial rapid increase on water loss and solids gain was observed followed by a reduction in these flows close to the end of the osmotic process. Similar result is also reported by Kolawole et al., 2007. High level of water loss is reported for the ultrasound treated watermelon rind. This result can be attributed to an alteration in the structure of watermelon rind. This could be due to the tissues allowed more water movement from the tissue to the sugar solution with sonication treatments. The cellular tissue exposed to thermal or physical pretreatment loses cell wall rigidity, as well as inter cellular adhesion. Such changes in physical properties resulted in high solute uptake. Similar findings were reported by Leyva *et al*., 2007, who showed that ultrasonic treatment in bell pepper immersed in osmotic solution produced significant water loss.

Water loss and solid gain increased with the higher concentration of osmotic solution concentration. Watermelon rind immersed into 60°Brix solution showed higher water loss and solid gain compared to those immersed in 50°Brix and 40°Brix solutions. The increase on solid gain and water loss with the solution concentration is due the high concentration difference between the watermelon rind and osmotic solution, which increased the rate of diffusion of solute and water exchange with osmotic solution. Increased solution concentration resulted in increase in the osmotic pressure gradients and higher water loss (Kolawole et al., 2007).

During the stipulated immersion time, the water loss increased from 0 to a minimum of 42% for the watermelon rind treated for 10 min under ultrasound and subsequently immersed in

40°Brix solution and to a maximum of 81% for the watermelon rind treated for 30 min under ultrasound and immersed in 60°Brix solution for 6 h. Similar result is reported by Fernandes, 2008b, on ultrasound as pretreatment for drying pineapple. Ultrasound treatment increases water diffusivity due to the formation of microscopic channels which also offers lower resistance to water diffusion Fernandes *et al*., 2008a.

The surface plots showing the effect of the immersion time, treatment period and sugar concentration on water loss of conventionally treated watermelon rind are shown in the Fig. 2. It is clearly seen from the figure that, the water loss in conventionally treated watermelon rind was high at enhanced treatment period and immersion period irrespective of the sugar concentration.

It is evident from the figure that in general there was a gradual and steady increase in the water loss was noticed in the conventionally treated watermelon during the immersion period, but significant treatment effect was evident. It is obvious that the highest water loss (73 per cent) was recorded in the watermelon rind conventionally treated for 3 min and immersed in 60°Brix solution for 6 h and it was followed by the similar treatment but for 2 min conventional treatment. The same trend was noticed for ultrasound treatment as well.

The minimum water loss of watermelon rind (31%) was observed for the conventional treatment of 1 min and immersion in 40°Brix solution for 1 hr. The ultrasound treated watermelon rind for 10 min showed 28.8% increase in water loss immersed in 60°Brix at 50°C after 6 h of immersion period, than the conventionally treated water melon rind for 1 min. Similar increase in water loss is also found for all other treatment.

3.2.Effect of sucrose concentration and immersion time on solid gain

Figures 3 and 4 shows the surface plot of the effect of sucrose concentration, different treatment period and immersion period on the solid gain at 50°C treated with ultrasound and conventional methods, respectively. The rate of solid gain in ultrasound and conventional treatments is less than the water loss for the same treatments. The rate of increase in solid gain decreased with the increase in the immersion period. Increase in the ultrasound treatment duration increased the rate of solid gain. Higher the concentration of sugar higher is the rate of solid diffusion. Watermelon rind immersed in 60°Brix after treating in ultrasound for 30 min showed 15.3% and 23.9% increase in solid gain over the watermelon rind immersed in 50°Brix and 40°Brix, respectively after 6 h of immersion period. Conventionally treated watermelon rind showed comparatively higher gain in solids than ultrasound treated.

In the ultrasonic-assisted osmotic dehydration the watermelon rind gained 18% of sugar after 1 h immersion in 60°Brix solution, with ultrasound treatment for 30 min and in the osmotic dehydration without ultrasound application but conventionally cooked for 3 min, the watermelon rind gained 29% of sugar in the same period. The solid gain increases rapidly with immersion time where as it increases slowly with sucrose solution.

A second order regression equation was fitted to the experimental data and the following second order regression models were obtained after deleting the non-significant terms using the software Essential Regression (Equations 3-6).

The mean values of water loss and solid gain obtained for all the treatments were statistically analyzed and the ANOVA table is presented in the Table 1 and Table 2 respectively. From the analysis, it was found that the effect of operating condition, treatment period and immersion time were found to be highly significant ($P \leq 0.01$). The interactions between all the parameters were also found to be highly significant.

$$WL_{(Ultrasound)} = -10.98 + 0.405BS - 0.04495BS^2 + 0.03133BT + 0.118TS - 0.000578BT^2 \ (R^2 = 0.914) \quad (3)$$

$$WL_{(Conventional)} = -1183 + 0.09988BTS - 0.677TS^2 + 7.827S + 0.319BT - 0.0869BT^2 (R^2 = 0.952) \quad (4)$$

$$SG_{(Ultrasound)} = 0.229 + 0.142BS - 0.01217BS^2 + 0.00321ST^2 + 0.00005948BT^2 (R^2 = 0.975) \quad (5)$$

$$SG_{(Conventional)} = 2.857 + 0.07526BTS - 0.372TS^2 + 2.711S \ \ (R^2 = 0.952) \quad (6)$$

where: B is the sugar concentration, °Brix; T is the treatment period, min; S is the immersion period, n h; SG is the solid gain, %; WL is the water loss, %.

Table 1. Analysis of variance for water loss in watermelon

Source	DF	Sum of Squares	Mean Squares	F-ratio	SED	CD (0.05)	CD (0.01)
Treatment	125	187646.571429	1501.172571	276.1281**	--	--	--
Operating condition (O)	5	30638.857143	6127.771429	1127.1521**	0.41544	0.81820	1.07835
Treatment period (T)	2	18432.142857	9216.071429	1695.2190**	0.29376	0.57855	0.76251
Immersion period (S)	6	125713.904762	20952.317460	3854.0029**	0.44872	0.88375	1.16475
O x T	10	2289.000000	228.900000	42.1042**	0.71956	1.41716	1.86776
T x S	12	3358.523810	279.876984	51.4810**	0.77721	1.53070	2.01741
O x S	30	5967.809524	198.926984	36.5909**	1.09914	2.16474	2.85305
O x T x S	60	1246.333333	20.772222	3.8209**	1.90377	3.74944	4.94162
Error	252	1370.000000	5.436508	1.0000	--	--	--
Total	377	189016.571429	501.370216	92.2228	--	--	--

CV = 5.84 per cent; **-Significant at 1per cent level

Table 2. Analysis of variance for solid gain in watermelon

Source	DF	Sum of Squares	Mean Squares	F-ratio	SED	CD (0.05)	CD (0.01)
Treatment	125	66543.886243	532.351090	131.0936**	--	--	--
Operating condition (O)	5	6049.346561	1209.869312	297.9352**	0.35905	0.70714	0.93198
Treatment period (T)	2	8763.021164	4381.510582	1078.9648**	0.25389	0.50002	0.65901
Immersion period (S)	6	46985.423280	7830.903880	1928.3920**	0.38782	0.76380	1.00666
O x T	10	608.661376	60.866138	14.9885**	0.62189	1.22480	1.61424
T x S	12	2407.386243	200.615520	49.4024**	0.67172	1.32294	1.74358
O x S	30	1320.005291	44.000176	10.8352**	0.94995	1.87092	2.46580
O x T x S	60	410.042328	6.834039	1.6829**	1.64537	3.24052	4.27088
Error	252	1023.333333	4.060847	1.0000	--	--	--
Total	377	67567.219577	179.223394	44.1345	--	--	--

CV = 9.29 per cent; **-Significant at 1per cent level

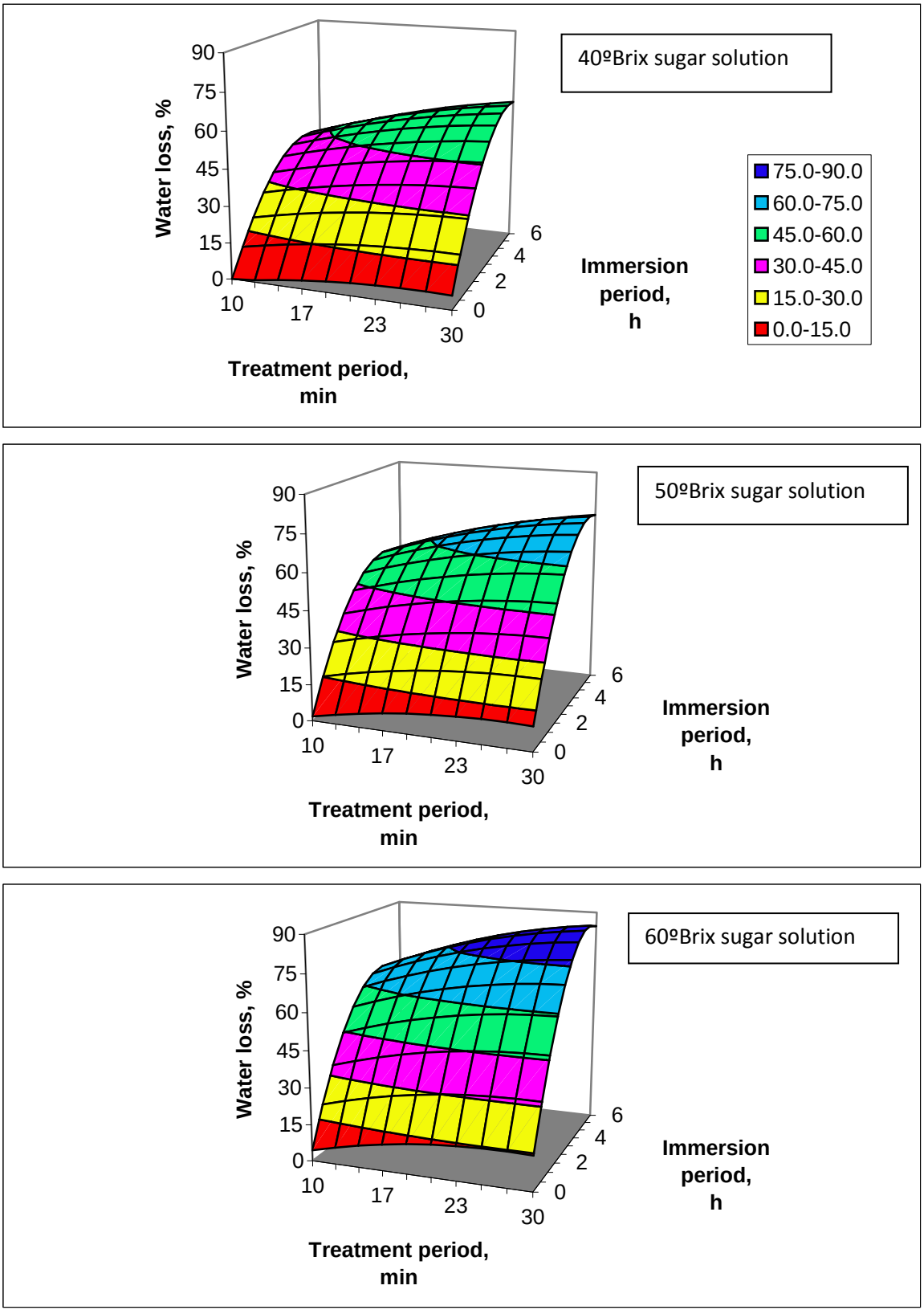

Figure 1. Surface plots of water loss in watermelon rind subjected to ultrasound pretreatment

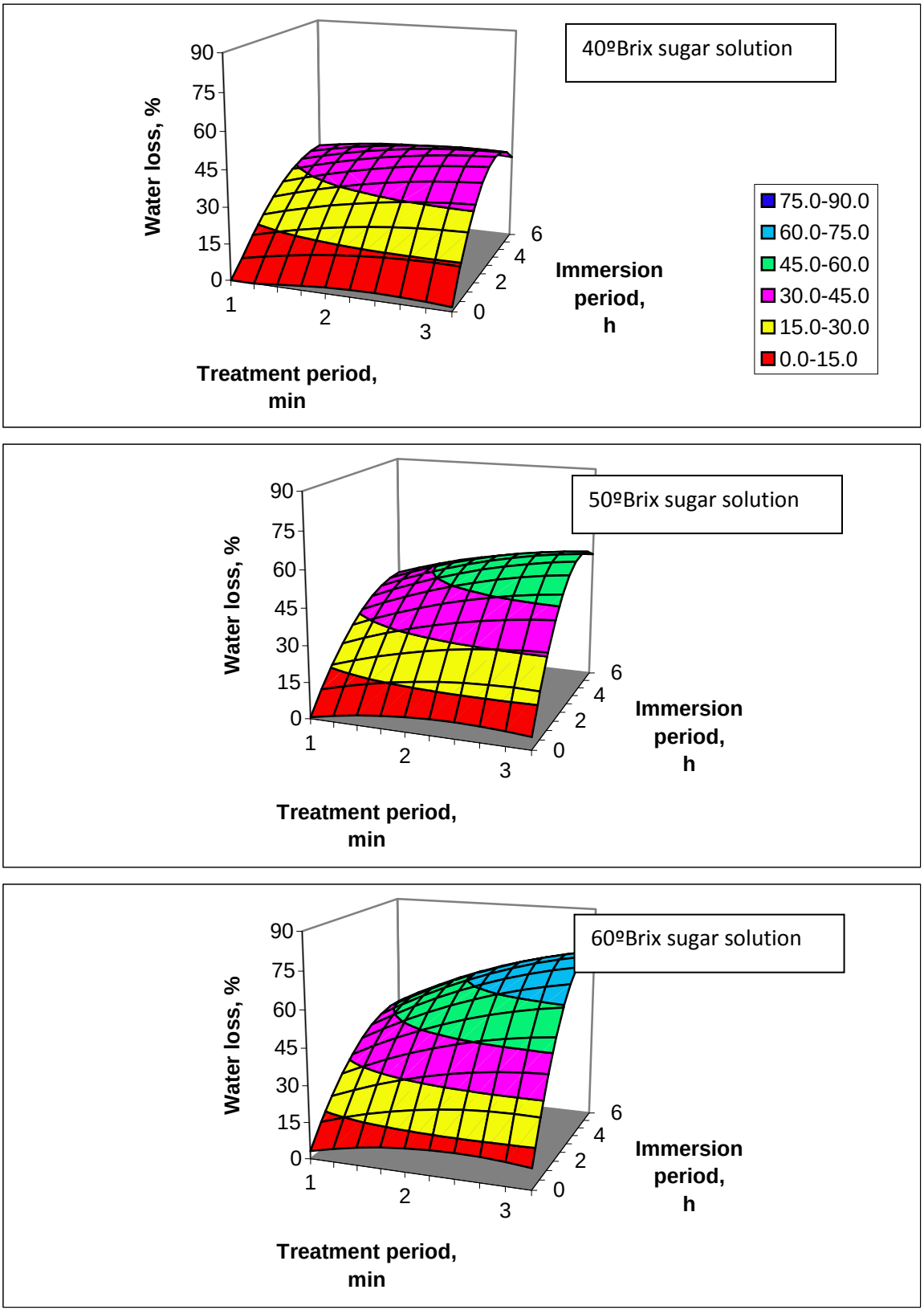

Figure 2. Surface plots of water loss in watermelon rind subjected to conventional pretreatment

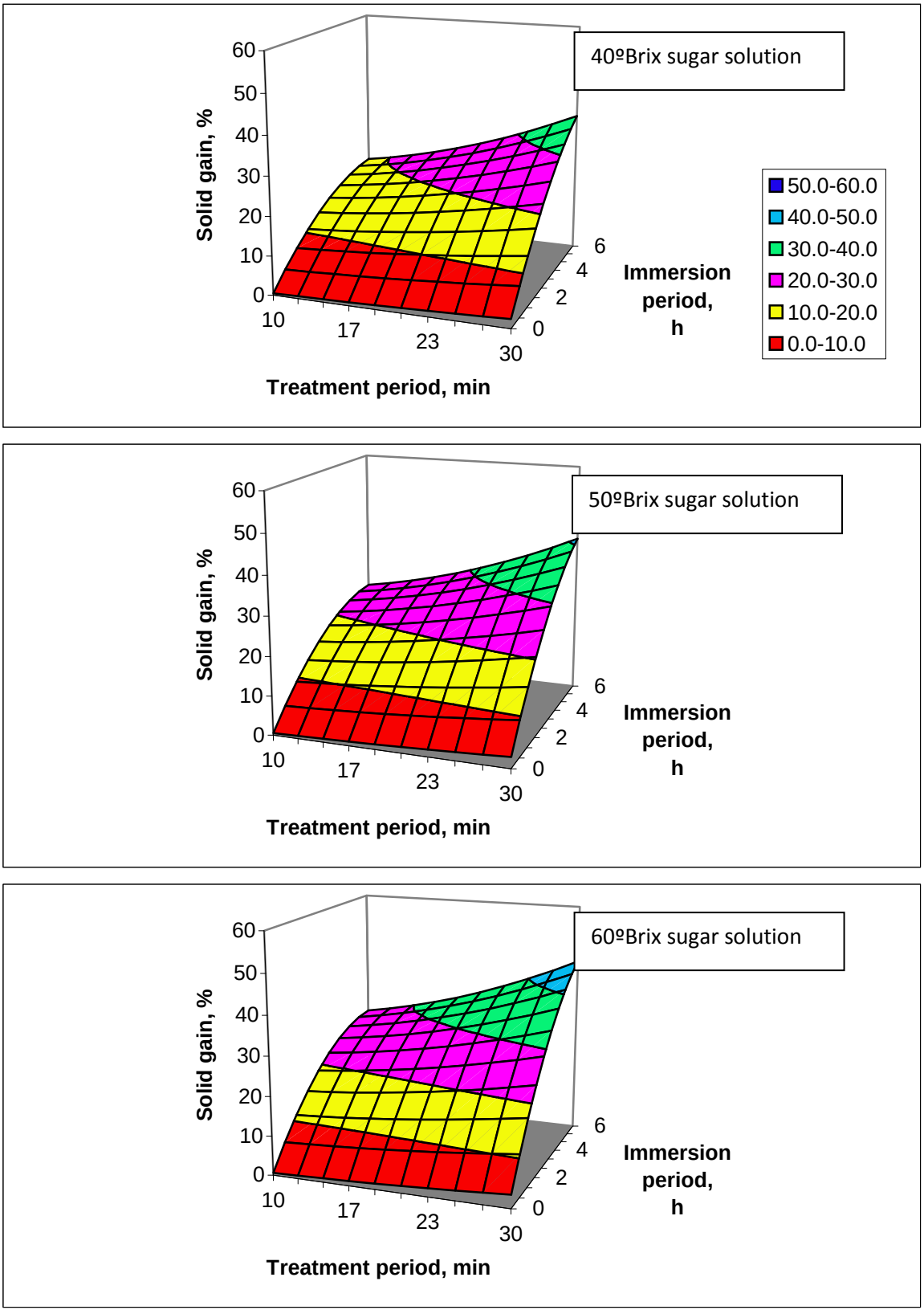

Figure 3. Surface plots of solid gain in watermelon rind subjected to ultrasound pretreatment

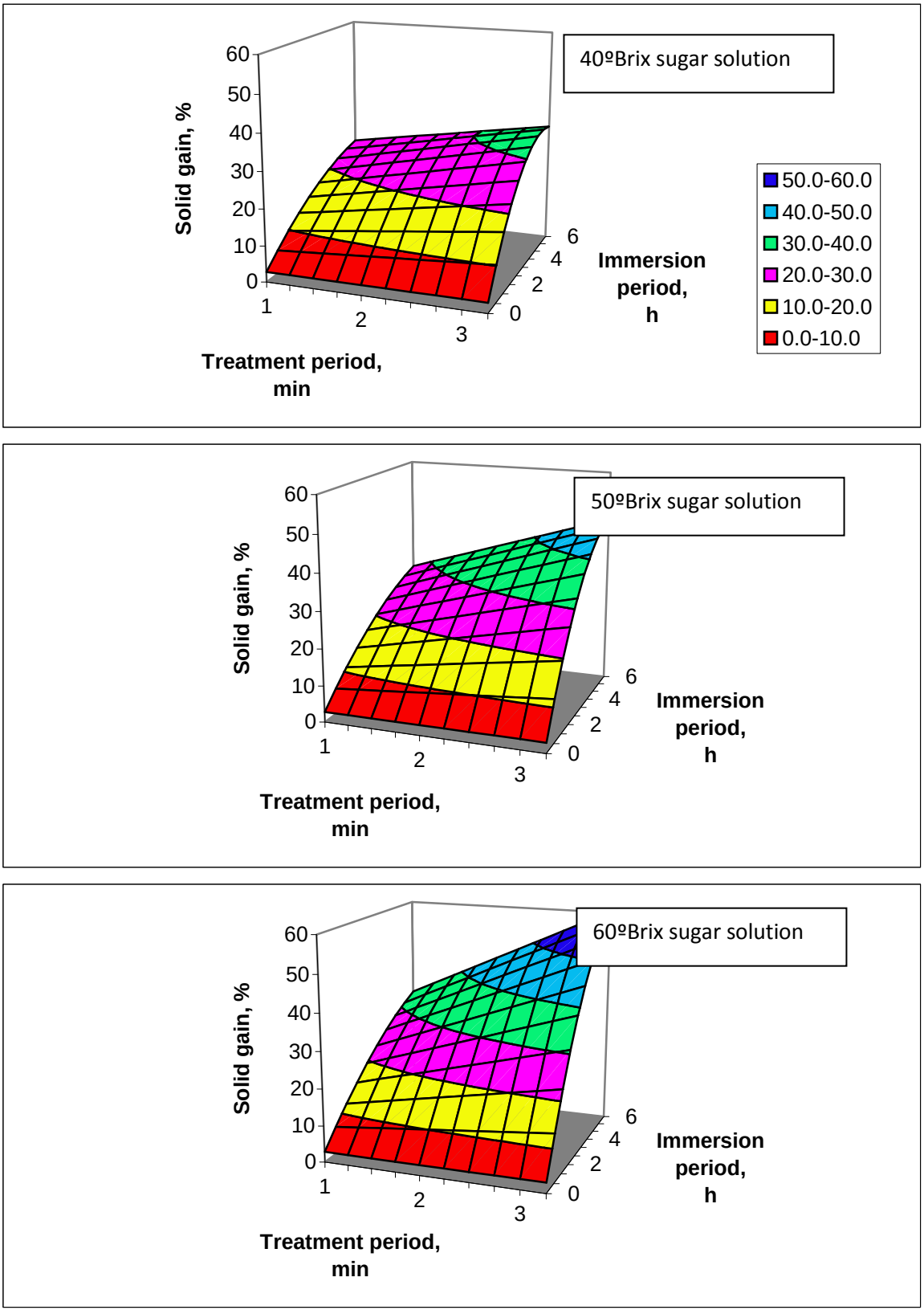

Figure 4. Surface plots of solid gain in watermelon rind subjected to conventional pretreatment

The analysis of variance (ANOVA) was carried out for the second order regression models and it was found that the model was significant at 1% level. High values of R^2, obtained for water loss during both ultrasound and conventional method, indicate good fit of experimental data.

The F-test of the above models was found to be significant from the analysis. Hence the developed model was adequate to describe the relationship of various process parameters with respect to water loss in watermelon. The regression coefficients in the models were tested for significance with t-statistics and it was observed that the interaction terms *viz*., BS, BS^2, BT, TS and BT^2 significantly influenced the model with respect to water loss and solid gain in ultrasound treated watermelon rind and the linear term immersion period (S) and interaction terms *viz*., BTS, TS^2, BT, BT^2 and BS^2 significantly influenced the model with respect to water loss in conventionally treated water melon.

4. Conclusions

The results showed that ultrasonic treated watermelon rind could have 37.93% less sugar than the conventionally processed watermelon rind. As such, the ultrasonic treatment may be an interesting process to produce low sugar dried fruits.

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PHYSICOCHEMICAL PROPERTIES AND STABILITY OF MELANIN FROM *AURICULARIA AURICULA* FERMENTATION BROTHS

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ABSTRACT

Physicochemical properties and stability of melanin from *Auricularia auricula* fermentation broths were studied. The result showed that *A. auricula* fermentation broths melanin powder was dark with a little red and yellow colored (L^*= 38.88, a^*= 0.36, b^*= -0.13). *A. auricula* fermentation broths melanin was insoluble in both water and common organic solvents. It dissolved only in alkali aqueous solution and precipitated in acidic aqueous solution (pH<3). *A. auricula* fermentation broths melanin was gradually oxidative bleached by oxidant and was stable to reducer. It exhibited strong optical absorbance in a wide UV-VIS spectral range. Furthermore, *A. auricula* fermentation broths melanin was relatively stable in the range of 20-100 °C and under nature light. Result of present study indicated that *A. auricula* fermentation broths melanin could potentially be used in the food industry as a natural colorant.

1. Introduction

Melanin is a dark-colored polyphenolic pigment produced from oxidative polymerization of phenolic or indolic compounds by tyrosinase (Chen et al., 2008). These natural pigments are synthesized by some fungi, plants, animals and several bacterial species (Dalfard et al., 2006). Melanins from different sources possess similar physicochemical properties, including strong light absorbance, unusual solubility and remarkable redox properties. In addition, melanin has a number of healthful functions, such as antioxidation (Hung et al., 2002; Różanowska et al., 1999; Tu et al., 2009), anti-HIV activity (Manning, et al., 2003; Montefiori and Zhou, 1991) and immunomodulatory activity (Sava et al., 2001). These functions promise natural melanin with great development potential as a healthful food colorant.

Auricularia auricula is a precious macro-fungus distributed in the Northeast Provinces of China and has been used as food and drug in China for a long time (Zhang et al., 1995). *A. auricula* fruiting bodies, a kind of edible black-brown mushroom, are rich in melanin and are increasingly popular as a "black food" in China. Melanin is considered to be one of the most important functional components in these "black food" (Tu et al., 2009). A large amount of melanin could also be produced by *A. auricula* through submerged culture. However, there is little information available about physicochemical properties and stability of melanin from *A. auricula* fermentation broths in current reports. In this study, melanin was isolated and purified from *A. auricula* fermentation broths and its physicochemical properties and stability were investigated.

2. Materials and methods

2.1. Materials

The fungal strain *A. auricula* RF201 was purchased from the Institute of Edible Mushroom of Jiangsu Academy of Agricultural Sciences (Jiangsu Province, China). Stock cultures were maintained on potato dextrose agar (PDA) slants and subcultured every two months. Slants were inoculated with mycelia and incubated at 25 °C for 7 d. All the chemicals and reagents used in the experiment were of analytical grade.

2.2. Preparation and purification of melanin

For production of the inoculum, the strain was transferred to seed medium (potato dextrose broth medium) with 6 mm diameter discs from PDA plates. Four discs were inoculated to 50 mL liquid medium in an Erlenmeyer flask (250 mL) and then incubated at 25 °C on a reciprocating shaker (100 rpm) for 5 d. The inoculum (10%, v/v) was transferred into an Erlenmeyer flask (250 mL) containing 50 mL of fermentation medium consisted of 30% (v:v) wheat bran extract, 0.4% (w:v) casein, 0.2% (w:v) L-tyrosine, 0.1% (w:v) copper sulfate, 0.1% (w:v) magnesium sulfate, 0.1% (w:v) potassium dihydrogen phosphate and 0.01% (w:v) vitamin B_1. Afterward, it was cultivated at 25 °C for 5 d at the pH 8.0 and rotation time of 100 rpm.

Purification of melanin was performed as described by Wu et al. (2008) with proper modification. Fermentation medium was centrifuged (4000 rpm, 5 min) and filtered to remove mycelia and insoluble particles. The pH value of the supernatant was adjusted to 2.0 with 3 M HCl to precipitate melanin, followed by centrifugation at 10000 rpm for 20 min and pellet collection. The pellet was washed with chloroform, ethyl acetate and ethanol for three times. Finally, the purified melanin was lyophilized and stored at -20 °C.

2.3. Visual color of melanin

Visual color of melanin powder was measured using a Minolta colorimeter (CR-400, Minolta Camera Co. Ltd., Osaka, Japan) with the Hunter Lab color system. The color values were expressed as *L** (whiteness or brightness/darkness), *a** (redness/greenness) and *b** (yellowness/blueness).

2.4. Solubility of melanin

The melanin (100 mg) was added to 10 mL of water, aqueous acid, alkali, or common organic solvents (such as ethanol, methanol, chloroform, acetone, aether, petroleum ether, benzene, ethyl acetate and butanol) under stirring for 1 h, and stood for 0.5 h, and then filtered. The absorbance of solutions was recorded at 400 nm in a UV-2802 diode array spectrophotometer (Unico Instrument Co. Ltd., Princeton, NJ, USA) to attain the solubility of melanin (Wang et al., 2006).

2.5. Redox properties of melanin

Redox properties of melanin were measured according to the basic procedure designed with minor modifications (Wang et al., 2006). Ten milliliter of 100 mg/L melanin solutions and 50 mL of different concentrations of $KMnO_4$, $K_2Cr_2O_7$, H_2O_2, NaOCl and Na_2SO_3 were mixed, and then the homogenate absorbance was determined at 400 nm.

2.6. UV-VIS absorption spectrum of melanin

Melanin was dissolved in alkaline distilled water (pH 10.0) at final concentration of 20 mg/L. The UV-VIS absorption spectrum of the melanin solution was scanned using a UV-2802 diode array spectrophotometer at wavelengths ranging from 200 to 800 nm.

2.7. Effect of temperature on stability of melanin

Melanin was dissolved in alkaline distilled water (pH 9.0) at final concentration of 50 mg/L. The heat stability of melanin solution was measured after treatment in a thermostatically controlled bath at 25, 50, 75 and 100 °C. The samples were held at each temperature for specific times and then cooled immediately in an ice bath. Subsequently the

absorbance of the solutions was recorded at 400 nm to attain the heat stability of melanin.

2.8. Effect of light on stability of melanin

The 50 mg/L melanin solution (pH 9.0) was held under natural light or at dark place for specific time and the absorbance was determined at 400 nm to attain the light stability of melanin.

3. Results and discussions

3.1. Visual color

Color values of *A. auricula* fermentation broths melanin are shown in Table 1. Results from the colorimeter indicated that melanin presented lower *L** value (38.88), *a** value (0.36) and *b** value (-0.13) in Hunter Lab color system. This indicated that melanin powder was dark with a little red and yellow colored.

3.2. Solubility

As is shown in Table 2, the solubility experiments indicated that *A. auricula* fermentation broths melanin was insoluble in both water and all common organic solvents (such as ethanol, methanol, chloroform, acetone, aether, petroleum ether, benzene, ethyl acetate and butanol). It dissolved only in alkali aqueous solution and precipitated in acidic aqueous solution (pH<3). The solubility of *A. auricula* fermentation broths melanin was very similar to those of natural melanin previously reported and synthetic melanin (Babitskaya et al., 2000; Tu et al., 2009).

3.3. Redox properties

The experimental results showed that *A. auricula* fermentation broths melanin exhibited marked redox properties (Table 2). They were gradually oxidative bleached by $KMnO_4$, $K_2Cr_2O_7$, H_2O_2 and NaOCl, illustrating that *A. auricula* fermentation broths melanin could be decolorized by strong oxidant. However, the absorbance of the melanin remained almost unchanged in Na_2SO_3, indicating that melanin was stable to reducer. These results revealed that *A. auricula* fermentation broths melanin presented the same redox properties of natural melanins previously reported (Chen et al., 2008; Suryanarayanan et al., 2004).

3.4. UV-VIS absorption spectrum

The UV-VIS spectrum (200-800 nm) of *A. auricula* fermentation broths melanin is shown in Figure 1. Melanin absorbed strongly in the UV region and progressively less as the wavelength increased. According to a previous report (Riley, 1997), strong optical absorbance in a wide spectral range was one of the most conspicuous properties of melanin due to the high degree of conjugation in the molecule. Some melanins extracted from plant and animal, such as *Osmanthus fragrans*' seeds (Wang et al., 2006) and black-bone silky fowl (Tu et al., 2009), exhibited an additional shoulder at wavelength 260-280 nm. However, *A. auricula* fermentation broths melanin had no evident shoulder in the 260-280 nm region, which was similar to those of the melanin extracted from *Hypoxylon archeri* (Wu et al., 2008), *Phyllosticta capitalensis* (Martinez et al., 2001) and *Pleurotus cystidiosus* (Selvakumar et al., 2008) of microorganisms, and synthetic melanin. It was well known that normal proteins had an absorption maximum at about 280 nm. Therefore, *A. auricula* fermentation broths melanin did not contain the protein.

3.5. Heat stability

The effect of temperature on *A. auricula* fermentation broths melanin stability was determined to ascertain the potential use of the melanin as a natural colorant. The brown color of the melanin was almost unchanged during heating. As is shown in Table 3, the quantitative data showed that the melanin loss was only 13% when the melanin solution was heated at 100 °C for 4 h. These revealed that *A. auricula* fermentation broths melanin was relatively stable to temperature.

Table 1. Color values of *A. auricula* fermentation broths melanin

Index	***L****	***a****	***b****
Color values	38.88±0.31	0.36±0.04	-0.13±0.02

The values are mean ± SD of three independent determinations.

Table 2. Solubility and redox properties of *A. auricula* fermentation broths melanin

Tests	**Response results**
Solubility in water	N
Solubility in organic solvents	N
Solubility in alkali aqueous solution	P
Precipitation in acidic aqueous solution (pH<3)	P
Reaction with $KMnO_4$	P
Reaction with $K_2Cr_2O_7$	P
Reaction with H_2O_2	P
Reaction with NaOCl	P
Reaction with Na_2SO_3	N

P: positive response; N: negative response.

Table 3. Heat stability of *A. auricula* fermentation broths melanin

Temperature (°C)	**Absorbance**				
	0 h	**1 h**	**2 h**	**3 h**	**4 h**
25	0.918±0.003	0.900±0.004	0.891±0.003	0.888±0.004	0.881±0.010
50	0.925±0.004	0.887±0.008	0.874±0.007	0.867±0.011	0.858±0.010
75	0.926±0.006	0.878±0.009	0.868±0.001	0.849±0.005	0.838±0.009
100	0.918±0.003	0.863±0.018	0.845±0.012	0.813±0.014	0.804±0.009

The values are mean ± SD of three independent determinations.

Table 4. Light stability of *A. auricula* fermentation broths melanin

Light source	**Absorbance**			
	0 d	**1 d**	**3 d**	**5 d**
Dark	0.921±0.006	0.921±0.005	0.917±0.008	0.915±0.008
Nature light	0.919±0.006	0.916±0.008	0.909±0.015	0.902±0.021

The values are mean ± SD of three independent determinations.

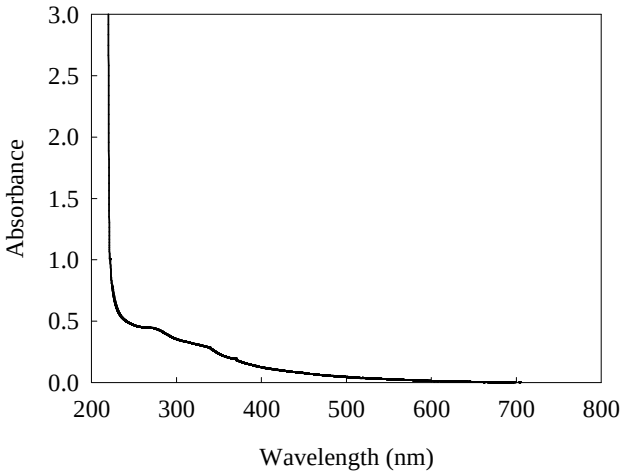

Figure 1. UV-VIS spectrum of *A. auricula* fermentation broths melanin.

3.6. Light stability

Table 4 showed that the absorbance of *A. auricula* fermentation broths melanin solution slightly declined when put under natural illumination. After being treated for 5 d, the absorbance of the melanin decreased slightly with a loss of 0.017. And the color of the melanin solution had no obvious change. These indicated that light had less effect on melanin, which agreed with results reported by Wang et al. (2006).

4. Conclusions

In the present study, *A. auricula* fermentation broths melanin was prepared and its physicochemical properties and stability were investigated. Melanin powder presented lower values (*L**, *a** and *b**) in visual color. *A. auricula* fermentation broths melanin was insoluble in both water and common organic solvents. It dissolved only in alkali aqueous solution and precipitated in acidic aqueous solution (pH<3). Melanin was gradually oxidative bleached by oxidant and was stable to reducer. It exhibited strong optical absorbance in a wide UV-VIS spectral range. Melanin was relatively stable to temperature and light. *A. auricula* fermentation broths melanin could be further used as a natural food colorant.

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THE STUDY ON SAFETY ASSESSMENT OF DAILY ARSENIC INTAKE OF VARIOUS RESIDENT POPULATIONS IN GUANGZHOU CITY

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ABSTRACT

Arsenic concentration of food from Guangzhou city in China was determined by hydride generation atomic fluorescent spectrometry. A wide range of arsenic concentration (0.0178 - 0.2226 $mgkg^{-1}$) was found among the various food. The arsenic concentration of animal foods was obvious higher than that of plant foods, particularly organ meat, aquatic productions, rice and pork were main resources of arsenic. The obvious different daily arsenic dietary intake in different residents was reported: high income groups (95.39 μgd^{-1})>middle income groups (90.53 μgd^{-1}) > low income groups (86.62μgd^{-1}), the daily arsenic dietary intake of the elder was 59.70 μgd^{-1}, the child was 40.23 μgd^{-1}, university man was 96.30 μgd^{-1}, the worker was 89.05 μgd^{-1}, the standard man was 94.46 μgd^{-1}. Rice, vegetable and animal foods were the major contributor to dietary intake. The daily arsenic dietary intake of resident was safety in Guangzhou.

1. Introduction

Arsenic is a major public health concern worldwide. World Health Organization (WHO) and the United States Environmental Protection Agency (EPA) think arsenic (As) as a known carcinogen. International Agency for Research on Cancer (IARC) confirms arsenic class I carcinogen on human (Argos, 2015). Arsenic has a reputation as a poison, because arsenic trioxide was used during medieval times as an agent for murder. Toxicity is a property of a specific compound and varies with the composition and structure. Developments in analytical methodology made it possible not only to determine total arsenic but also the various arsenic compounds in a variety of matrices The main cause of arsenic pollution in the environment is the high concentration of arsenic in soil parent material, and the use of industrial manufacturing, mining and agricultural pesticides, disinfectants, fungicides and herbicides (Mirna et al., 2015, Robberecht et al., 2004). The soil, drinking water and food are subjected to serious pollution of heavy metal in China, the arsenic risk to human health have been more and more attention, and the study found groundwaters with arsenic concentrations higher than the WHO provisional guide value of 10 μg/L are found in many parts of the world (Yu et al., 2009; Wang et al., 2004; Liu et all., 2004; Kar et al., 2013; Carmen et al. 2015). Research shows that China is one of the countries with most serious arsenic pollution. In Xinjiang, Inner Mongolia, Hunan, Guangdong province and some have been the emergence of endemic arsenic poisoning. Xiao Xiyuan found the arsenic concentration of major grain and oil crop of

32.2% and 34.8% in arsenic polluted area higher than tolerance limit of arsenic in food for China (Xiao et al., 2009). The current study pays more attention to the regional environmental arsenic pollution and arsenic concentration of food, combined with the research on human health is relatively less.

In this paper, the comprehensive investigation of the arsenic concentration of market food, combined with dietary survey results of various resident populations, and assessed the dietary arsenic health risk and the main way of arsenic intakes of the residents in Guangzhou city. The results provide scientific basis for dietary health and arsenic pollution controlling in China.

2. Materials and methods

2.1 Sample preparation

Sampling sites located in the wholesale markets, farmers markets and super markets which sell most of the vegetables, aquatic products and fruits in Guangzhou. The farmers markets located in the different administrative region of Guangzhou. The supermarkets are a relatively large market food sale. Random samples were collected in each sampling site. Acquisition with a sample 3-5 at each sampling location, and then mixed into a sample. Samples were frozen immediately on laboratory refrigerator after being collected. A total of 109 kinds of samples, and have 830 samples.

2.2 Arsenic analysis

Method: the samples were digested in nitric acid and perchloric acid mixture. Samples were analyzed by atomic fluorescence spectrometer (GB/T5009.11-1996).

Measuring instrument: double channel atomic fluorescence spectrophotometer AFS-610 (Beijing Titan Instruments Co. Ltd.).

Quality control: the environmental standard samples provided by National Standard Substances Center (GBWZ19001-94 bovine liver) and recovery determination.

2.3 Dietary survey

Dietary survey methods of this study included the accounting method, survey method and weighing method. This study investigated 450 university students (13-31 years) of 5 colleges and universities, 541childred (3-6 years) of 10 kindergartens, 427 old peoples (62-70 years) of 8 nursing homes, and 601workers (18-51 years) of 5 factories and 2 building sites. Investigation of dietary of standard man and different income groups used the data of the nutritional status of residents in Guangdong province in 2002. The standard man was adult male engaged in light physical activity. Low income group referred to the people who hers average annual family income was less than 2000 yuan. Middle income group referred to the people who hers average annual family income was 2000-10000 yuan. High income group referred to the people who hers average annual family income was more than 10000 yuan (Ma et al., 2005).

2.4 Statistical analysis

Food classification method according to "Chinese food composition table in 2002". Data analysis and statistical methods are Excel and SPSS In this study, calculation of arsenic dietary intakes of the same type food (such as vegetables), for which different types (such as leafy vegetables, tubers and other) food, are corrected according to account for in the dietary ratio. Calculating method is showed as formula 1.

$$EDA = I_i = \sum C_{ik} \cdot N_k \cdot D_j \quad (1)$$

where: I_i is the As daily intake; C_{ik} is the As concentration of the K food; D_j is the Daily consumption of j food; N_k is the K food proportion of j food in the diet (weight).

3. RESULTS AND DISCUSSION

3.1 The Arsenic Concentration of Food

As shown in Table2, the food of higher arsenic concentration is mainly organ meat and aquatic products, 0.2226±0.0779 $mgkg^{-1}$ and 0.2022±0.0810 $mgkg^{-1}$respectively.

Table 1. Percent recovery of arsenic of various food groups

Sample	Percent of recovery (%)		
	n	Range	Mean±S.D.
Rice	5	91.8-104.6	96.5±4.1
Soybean	5	90.8-107.4	95.0±4.7
Beef	5	92.0-105.3	94.8±5.6
Ctenpharyngodon idellus	5	92.3-97.7	95.7±2.4
Palaemon carinicauda	5	91.4-104.7	95.4±4.9
Loligo chinensis	5	92.3 -98.1	95.7±3.0
Malus pumila	5	94.7-103.1	97.3±5.7
Musa nana	4	91.9-96.3	94.5±2.4
Ciltrullus lanatus	4	93.4-104.2	96.1±3.9

Table 2. The arsenic concentrations of various foods of Guangzhou ($mgkg^{-1}$)

Food item	n	Mean	S.D.	Range	CV%
Rice	25	0.1500	0.1120	0.0280-0.5161	74.7
Noodle	22	0.0496	0.0047	0.0050-0.2130	9.6
Dry legume	27	0.0910	0.0545	0.0032-0.1927	59.9
Legume products	13	0.0199	0.0249	ND-0.1248	125.1
Vegetable	250	0.0353	0.0280	ND-0.3172	79.5
Fruit	198	0.0178	0.0031	ND-0.0572	17.5
Pork	13	0.0733	0.0166	0.0351-0.1100	22.6
Other meat	22	0.0883	0.0097	0.0481-0.1321	11.0
Organ meat	11	0.2226	0.0779	0.0672-0.5925	35.0
Poultry	19	0.0940	0.0045	0.0443-0.1558	4.8
Milk and milk products	24	0.0397	0.0413	ND-0.1210	104.0
Egg and egg products	24	0.0508	0.0179	0.0007-0.1825	35.2
Aquatic products	208	0.2022	0.0810	0.0075-1.2017	40.1

Table 3. The comparison arsenic concentration of food in Chinese four regions with this study ($mgkg^{-1}$, fresh weight)

Food item	North 1st region	North 2nd region	South 1st region	South 2nd region	This study
Rice	0.320	0.284	0.385	0.638	0.1500
Dry legume	0.102	0.151	0.170	0.090	0.0910
Meat	0.127	0.124	0.111	0.183	0.1281
Eggs	0.124	0.124	0.117	0.142	0.0508
Aquatic products	0.104	0.130	0.698	0.176	0.2022
Milk and milk	0.078	0.069	0.110	0.099	0.0508
Vegetable	0.104	0.107	0.146	0.135	0.0353
Fruit	0.098	0.084	0.059	0.121	0.0178

Table 4. The daily arsenic intake of various resident populations in Guangzhou (μgd^{-1})

Food item	Standard man	Low income group	Middle income group	High income group	Old people	Child	University student	Worker
Rice	43.60	57.00	49.80	41.50	18.00	14.80	54.80	58.43
Noodle	2.38	0.6	1.57	2.53	2.45	0.67	2.24	0.66
Dry legume	0.03	0.07	0.04	0.04	0.02	0.01	0.02	0.07
Legume products	0.58	0.51	0.65	0.61	0.68	0.29	0.4	0.44
Vegetable	11.10	9.54	10.40	11.40	9.68	6.57	9.704	9.14
Fruit	1.25	0.67	1.07	1.45	0.90	0.82	0.90	0.50
Pork	8.98	5.23	7.98	8.95	6.44	3.36	7.89	5.84
Other meat	1.48	0.24	1.03	1.56	0.94	0.76	1.09	0.22
Organ meat	2.43	1.51	2.32	2.94	1.91	1.94	2.18	1.27
Poultry	5.40	1.98	3.85	5.89	3.79	2.20	3.98	2.93
Milk and milk products	1.54	0.11	0.59	1.95	0.62	1.01	0.73	0.12
Egg and egg products	1.40	0.69	1.00	1.52	1.30	0.62	0.89	0.71
Aquatic products	14.30	8.41	10.30	15.00	13.00	7.2	11.40	8.74
Total	**94.46**	**86.62**	**90.53**	**95.39**	**59.70**	**40.23**	**96.30**	**89.05**

Table 5. Dietary arsenic intakes of adult male in different areas (μgd^{-1})

Area	Study time	Dietary arsenic intake	TDMI (%)
This study	2004-2006	94.46	3.14
Spain (Egan, 2002)	2006	261.0	8.70
Mainland China (Li, 2006)	2000	276.1	9.20
American(Ysart, 2001)	1991-1996	58.1	1.94
England (Dabeka,1995)	1997	120.0	4.00
Canada (Urieta, 1996)	1985-1988	59.2	1.97
Basque (Tsuda, 1995)	1990	286.0	9.53
Japan (Haeng,2006)	1992	280.0	9.33
France (Li, 2006)	2000/2001	36.9	1.23
Australia (Li, 2006	1998	62.3-80.4	2.08-2.68
Korean (Michael, 2005)	1998-1999	38.5	1.28
Germany (Tripathi, 1997)	1998	291.0	9.70
Bombay (Jasenka, 1996)	1993-1994	100.0	3.30
Croatia (Roser, 2008)	1988-1993	81.9	2.73

The arsenic concentration is much higher than other types of food. The arsenic concentration of rice is second, 0.1500 ± 0.1120 mgkg^{-1}. The arsenic concentrations of dry beans, pork and poultry are the middle level, between 0.0733-0.0940 mgkg^{-1}. The food of lower arsenic concentration is noodles, vegetables, milk and milk products, eggs and egg products, between 0.0353-0.0508 mgkg^{-1}. The arsenic concentrations of legume products and fruit are the lowest of all food, 0.0199± 0.0249 mgkg^{-1} and 0.0178 ±0.0031 mgkg^{-1} respectively. In general, the order of arsenic concentration in all kinds of food: organ meat, aquatic products **>** rice **>** dry legume, pork, other meat, poultry **>** noodle, vegetables, milk and milk products, **>** eggs and egg products **>** bean products, fruit. Li Xiaowei (Li et. Al, 2006) studied arsenic concentration of food in some regions of China. According to the partition principle and methods of the total diet study, the country is divided into 4 regions: North 1st region, North 2nd region, South 1st region and South 2nd region. The arsenic concentration of food and dietary arsenic intake were researched in each region. It is seen from table 2, the arsenic concentration of aquatic products in Guangzhou is significantly higher than that of South 2nd region, North 1st region and North 2nd region. The arsenic concentration of meat food and northern region is difference, slightly higher than the south region, significantly below the Southern District two. The arsenic concentration of milk foods is similar to the northern regions, but significantly lower than the southern region. The arsenic concentration of other food is significantly lower than that of four regions. Especially the arsenic concentration of the plant foods (such as vegetables, fruits, grains) is different to that of four regions. Overall, compared with the study, market food of Guangzhou is not contaminated with arsenic. Compared with <Tolerance Limit of Arsenic in Food for China>, arsenic concentration of various types of food are in safe level.

3.2 Daily arsenic intake of various resident populations

Table 4 shows that there are great differences of the daily dietary arsenic intakes between **v**arious resident populations in Guangzhou, and the change range is 40.23-96.30 μgd^{-1}. In all the surveyed resident populations, the daily dietary arsenic intake of university student is the highest (96.30 μgd^{-1}) and that of child is the lowest (40.23μgd^{-1}), and also the difference is larger. There are some differences between daily dietary arsenic intakes of different income groups, but the difference is not big. The order of daily intake of various income resident populations shows as follows: high income groups (95.56 μgd^{-1})> middle income groups (90.53 μgd^{-1}) > low income groups (86.62 μgd^{-1}). Obviously, the dietary arsenic intakes of more income groups are higher. The main source of dietary arsenic intake of low income group is rice, significantly higher than that of high and middle income groups. Arsenic intake from other food was lower than other groups. It is obvious differences that the arsenic intake from the food of high concentration arsenic. Such as, the arsenic intake from pork, poultry, aquatic products and other food is much lower than other income groups.

Table 4 shows that dietary arsenic intake of old people is 59.70 μgd^{-1}, child is 40.23 μgd^{-1}, college students is 96.30 μgd^{-1}, workers is 89.35μgd^{-1}, and the standard is 94.46μg.d^{-1}. In the all resident populations, dietary arsenic intake of university student is the highest, and child is the lowest. Arsenic intake of university student from meat food is more than old people, workers and child.

Overall, the food of rice, vegetable and animal food is the main source of arsenic intake of all groups. Rice is the main food of all groups as dietary intake. Our results are

different from foreign, such as the United States, Canada, Australia and France, which the main source of dietary arsenic is seafood. Especially France, seafood for arsenic dietary source of contributions to 60% (Li et al., 2006). This is mainly due to differences of dietary structure between Chinese and western.

3.3. The Safety assessment of daily arsenic intake

Food and Agriculture Organization of United Nations (FAO) /WHO combine with Codex Committee announce that the Tolerance Daily Maxium Intake (TDMI) of total arsenic is 50 μgkg^{-1} body weight (BW), and calculate the intake is 3000 $\mu gd^{-1}$of the standard body weight 60kg. WHO also recommends Provisional Tolerable Weekly Intake (PTWI) of total arsenic is 15 gkg^{-1} BW, based on the standard man. The arsenic acceptable daily Intake (ADI) of adult is about 0.128 mgd^{-1} (Tsuda et al., 1995; Badal et al., 2002). In this study, the total arsenic intake by dietary of the standard man in Guangzhou is 3.14% of TDMI. The other people to TDMI in Guangzhou: the high income group is 3.17%, the middle income group is 3.02%, the low income group is 2.89%, the old people are 1.99%, the university student is 3.21%, and worker is 2.97%.

There are has few studies on dietary intake of child now. For evaluating child's dietary arsenic, we try to determine the daily dietary arsenic intakes due to child's weight. If the average weight of 3-6 years old child was 15-30 kg, the safe range of the daily dietary arsenic intakes of 3-6 years old child is 750-1500 μgd^{-1} according to TDMI of dietary arsenic. With such a criterion to evaluate, we think of child's dietary arsenic intakes in safe level in Guangzhou. Joyce thinks short-term and long-term arsenic exposure dose of 0-6 year old child should be less than respectively 0.015 $mgkg^{-1}$BW and 0.005 $mgkg^{-1}$ BW respectively (Joice et al., 2004). Comparison of the results, arsenic intake of 3-6 years old child is safety in Guangzhou.

Li Xiaowei's study thought that arsenic intake of Chinese residents from water accounted for a large proportion (about7%) (Li et al., 2006). In order to evaluation of arsenic intake of residents of Guangzhou, we consider the arsenic intake from water. China has formulated the relevant provisions of the sanitary standard of drinking water (the standards of drinking water health). The sanitary standard of arsenic concentration should not exceed 0.05 mgL^{-1}, and the standards of bottled purified drinking water health of arsenic concentration should not exceed 0.01 mgL^{-1}. About the characteristics of arsenic concentration of drinking water in Guangzhou, the relevant research and water quality testing report of Guangzhou that comply with the relevant standards, is generally believed that the arsenic concentration is 1/10 of the standard (Yang et al., 2001; Xu et al., 2005). Therefore, the drinking water volume of residents is 2L every day, at the same time, the arsenic content of water is 0.0025 mgL^{-1}, so the arsenic intake of residents of Guangzhou from drinking water is only 5 μgd^{-1}. Thus, even taking into account the arsenic intake from drinking water, arsenic intake of residents of Guangzhou is safety.

3.4 Comparison with other studies

The comparison of dietary arsenic intakes of adult male in different areas is shown in Table 5. Li Xiaowei (Li et al., 2006) found that daily dietary arsenic intakes of Mainland Chinese residents reached 276.1 μgd^{-1}, is 9.2% of TDMI. The result of this study is a larger difference, only for 3.14% of TDMI. Compared with other countries and areas, the dietary arsenic intakes of Guangzhou standard man higher than the United States, Canada, France, Australia, Korea, Croatia and other regions, significantly lower than the Spanish, British, Basque area, Japan and Germany, and had little difference than Mumbai.

4. Conclusions

(1) The order of arsenic concentration of food as follows: organ meat (0.2226±0.07791 μgkg^{-1}) > aquatic products (0.2022±0.0810 μgkg^{-1}) > rice (0.1500±0.1120 μgkg^{-1}) >

poultry (0.0940±0.0045 μgkg^{-1}) > dry legume (0.0910±0.0545 μgkg^{-1}) > other meat (0.0883±0.0097μgkg^{-1}) > pork (0.0733 ± 0.0166 μgkg^{-1}) > egg and egg products (0.0508±0.0179 μgkg^{-1}) > noodles (0.0496 ± 0.0047μgkg^{-1}) > milk and milk products (0.0397 ± 0.0413 μgkg^{-1}) > vegetable (0.0353±0.0280 μgkg^{-1}) > legume products (0.0199±0.0249 μgkg^{-1}) > fruit (0.0178±0.0031 μgkg^{-1}).

(2) There is obvious different of daily intake of arsenic among various resident populations. The order of daily intake of various income resident populations as follows: high income groups (95.56 μgd^{-1}) > middle income groups (90.53 μgd^{-1}) > low income groups (86.62 μgd^{-1}). The dietary arsenic intakes of more income groups are higher. Daily intake of other resident populations as follows: old people (59.70 μgd^{-1}), child (40.23 μgd^{-1}), university student (96.30 μgd^{-1}), worker (89.05 μgd^{-1}), standard man (94.46 μgd^{-1}).

(3) The rice was major contributor to the daily arsenic dietary intake of resident. Vegetable and animality food (pork, chicken and aquatic products) were the secondly contributor. Fruit, egg and egg products and legume and legume products and so on, were very little contributor.

(4) The daily intakes of arsenic of various resident populations of Guangzhou are safe to human health, but it is necessary to avoid arsenic accumulate contamination in food chain.

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UNIVERSITY-INDUSTRY EDUCATIONAL AND TRAINING INITIATIVES A REVIEW OF THE 1ST FOOD-STA VIRTUAL CONFERENCE ON 7-8 MAY 2015

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This conference was organized by the ERASMUS+ Knowledge Alliance project (554312-EPP-1-2014-1-AT-EPPKA2-KA, http://www.food-sta.eu). This project aims to establish an independent "**EuFooD-STA Centre**" as virtual platform with physical hubs in different countries as a legal and organisational frame for international and sustainable collaborations between industry and academia in the food sector.

The independent "EuFooD-STA Centre" will enhance innovative education & training for students, targeted towards the needs of the industry and thus increasing their employability and on the other hand innovative continual professional development (CPD) for academic and company staff by facilitating experience exchange.

These will be achieved by:

- work and training experiences through **industrial and university placement**
- promoting of **best practices** of already existing training materials and methods
- **joint** academic/company **tutoring** and **supervision**
- joint development of **guidelines** for the design and implementation of **joint "industrial master" curricula**
- joint development of a **CPD programme** and **certification scheme** for company and university staff
- joint development of **trainings material**, modules, courses, etc. to improve existing academic curricula and CPD training
- development of a **digital library** consisting of tutorials, recorded webinars, online courses, etc., a **multilingual database** and an **e-learning platform**

Industry partners can train their staff and make use of placement of researchers who can collect practical experiences. **University partners** can train their teaching staff, tune their study programmes, make use of modules from other Universities or new modules developed in collaboration with Industry and other academic partners. The independent "EuFooD-STA Centre" is open to other Universities and food companies who want to be involved without funding.

The aim of this conference was to collect best practices of university-industry educational and training initiatives for food study programmes and CPD (continual professional development). Speakers from universities, industries and research presented case studies and practices.

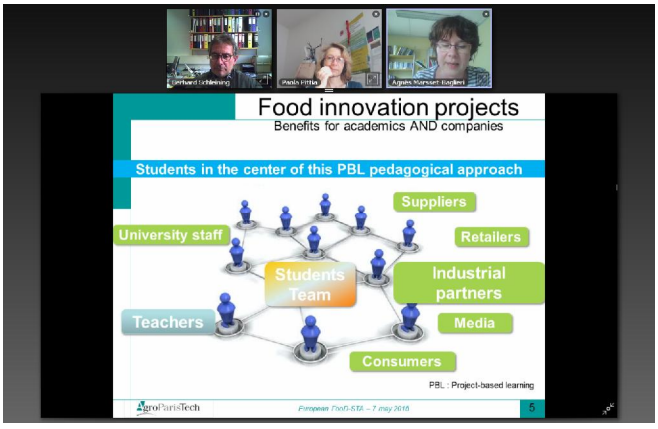
The conference was carried out as a virtual conference using the online tool GotoWebinar© (http://www.gotomeeting.com/webinar). The event was announced worldwide via the ISEKI-Food Association to more than 9000 subscribers of the ISEKI-News. From 56 registered persons 43 participated from 19 countries (**Austria, Brazil, Canada, Estonia, France, Germany, Ireland, Italy, Lithuania, Malaysia, Mexico, Portugal, Romania, Slovakia, Spain, Sri Lanka, Switzerland, Turkey, United Kingdom**). From the 32 abstract submission 17 contributions were selected for oral presentations and the rest as posters.

On the first day, after an introduction in the project and how to use the control panel of the webbased conference by the coordinator of the project **Gerhard Schleining** (BOKU, Austria), mainly **prespectives of the universities** were presented and discussed.

Paola Pittia (University of Teramo, Italy) spoke about challanges and opportunities to innovative education and training in food science and technology.

Eduardo L. Cardoso (Escola Superior de Biotecnologia, Portugal) talked about promoting entrepreneurship trough University-Industry cooperation.

Ermis Ertan (Istanbul Sabahattin Zaim University, Turkey) reported the views of Food Engineering students on the effects of industry-integrated learning on employability.



Chathudina Janitha (Liyanage Sabaragamuwa University of Sri Lanka) talked about current and planned pedagogical initiatives for enhancing university-industry interactions and fostering entrepreneurial skills.

Romina Zanabria (Canadian Research Institute for Food Safety (CRIFS), University of Guelph, Canada) presented the "Loblaw Academy Case Study" and how to measure the e-Learning success.

Gwenola Bertoluci (AgroParisTech, France) gave an overview on the Food eco-innovation training: Idefi-Ecotrophelia.

Henry Eric Spinnler (AgroParisTech, France) presented the Savencia Academy, an example of long-term collaboration with Industry for lifelong learning.

Susanne Braun (University of Hohenheim, Germany) finished with an overview on research, training and Food Study Programms in Hohenheim.

The second day was dedicated more to **perspectives of research and industry**. **Brian McKenna** (University College Dublin, Ireland) gave some personal views based on experiences at UCD.

Maria Ana Marques (Frulact, Portugal), an industrial partner of the project, spoke about the opportunities for university-industry cooperation as key to continuous improvement.

Cristina L.M. Silva (Escola Superior de Biotecnologia, Portugal) reported on the advantages of pilot plant and cooking lab facilities for training and education. **Sadio Ramos** (Politécnico de Coimbra, Portugal) presented innovative pedagogical and learning experiences.

Giurgiulescu Liviu (Technical University of Cluj Napoca, Romania) pointed out the differences between traditional and modern stimulation of creativity.

Victor Kuri (Plymouth University, UK) presented how academia could gain from Knowledge Transfer Partnerships between university and food SMEs.

Finally **Marc Dryer** (Nestlé, Switzerland) gave insight in the view from the R&D in the industry for talent development.

The programme, list of participants and all abstracts are available public at http://www.food-sta.eu/2015-05-07. The presentations and posters are available only for partners and members of the project after login.

There will be a continuation of collectiong and discussing best practices in a forum at http://www.food-sta.eu. Everybody who registers on the website will be able to upload an abstract and supporting pdf documents and to discuss other contributions with the authors.

For further information please contact office@food-sta.eu.

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COMMUNICATION OF SCIENTIFIC RESEARCH FOR STUDENTS IN THE FIELD OF FOOD ON 15 MAY 2015

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