STUDIES ON THE PRESERVATION OF KOKUM (*GARCINIA INDICA*) RIND JUICE AT AMBIENT STORAGE CONDITION

Abstract

The study aimed to determine the effect of different concentrations of sodium benzoate at ambient storage condition on kokum rind juice. For this experiment, Factorial Completely Randomized Design (F.C.R.D.) was used. During the investigation, the chemical parameter, microbial analysis and sensory qualities of kokum rind juice were studied. During the 3 months of ambient storage condition, analysis was conducted at the interval of one month. The chemical parameter like acidity ,TSS, reducing sugar ,total sugar, Anthocynins changed significantly. From the experiment it can be concluded that the kokum rind juice could be stored at 1500 ppm sodium benzoate concentration in a good acceptable condition at ambient storage condition.

Introduction

Kokum (Garcinia indica Choisy) is a major indigenous tree spice crop that originated and is grown in India's Western Ghats(Padhye et al. 2009). Maharashtra's South Konkan region, Coorg, Wynad, and Goa, and is found in evergreen and semi-evergreen woods as well as as a home garden tree (Subash Chandran, 2005). The tree grows extensively in the Konkan region of Maharashtra.

As for a chemical composition of Kokam fruit is concerned, it contains 80% moisture, 2% protein, 2.8% tannin, 5% pectin, 14% crude fiber, 4.1% total sugars, 1.4% fat, 2.4% pigment, 22%

hydroxy citric acid, 0.06% ascorbic acid 5.90% organic acid, 2.40% anthocyanins, 2.60% ash and 1% starch (Krishnamurthy et al.1982, Sayeed et al. 2009 and Nayak et al. 2010)

It exhibits various phytochemical properties like anti-ulcerogenic, cardio protective, anticancer, chemo preventive, free radical scavenging and anti-obesity effects. Garcinia known for medicinal properties is also used for curing piles, dysentery, tumour pains and cardiac problems. Even fat reducing tablets and capsules based on Garcinia are available in the market (Rasha et al., 2015)

Today, the preservation of fruit juice has become a business activity of great importance, and countries with plentiful fruit resources and a short harvest season place more emphasis on well-established storage to preserve fruit quality, extend its shelf life and preserve fruit juice for off season use (Tasnim et al. 2010) Fruit juice must be conserved in order to be used later in the creation of beverages with juice as the main ingredient. However, due to the high nutritional content of fruit juices, storage of fruit juices at room temperature without the addition of any chemical preservatives or pasteurisation is not feasible.

Fruit juices are refreshing and retain characteristic taste and aroma even after a few months of their preparation into a beverage. The kokum rind juice has a very short storage life. Generally, after extraction of juice from kokum it starts deteriorating. Kokum juice is generally utilized for the preparation of different products like RTS, syrup, jelly,solkadi etc. however, it has a very poor storage life therefore a systematic research work is needed to increase the storage life of kokum rind juice conversion into a different value-added product. It is highly necessary to study the storage life of kokum fruit juice which would be useful for value addition. The storage life of kokum rind juice could be extended by using chemical preservatives as well as low-temperature storage. It is highly necessary to extend the storage life of kokum juice with preservatives under different storage conditions.

The chemical preservation technique is used primarily to prevent microbial spoilage of fruit juices during storage, both in retail stores and in the consumer's home. Benzoic acid (E210) and sodium benzoate (E211) are approved food preservatives with an acceptable daily intake of 5 mg/kg body weight by the FAO/WHO due to their long history of safe use. Sodium benzoate, with its broad antibacterial activity, non-volatility and water solubility, is widely used as a preservative especially in coloured fruit drink. The sodium benzoate is effective against yeast, mold and bacteria. It is most suitable for use as an antimicrobial agent in food and beverages which naturally are in the pH range below 4.5 per cent, compared to other preservatives (Kaddumukasa et al. 2017).

Materials and Methods

The fruits required for conducting research were procured from the Department of Horticulture, College of Agriculture, kokum from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth,

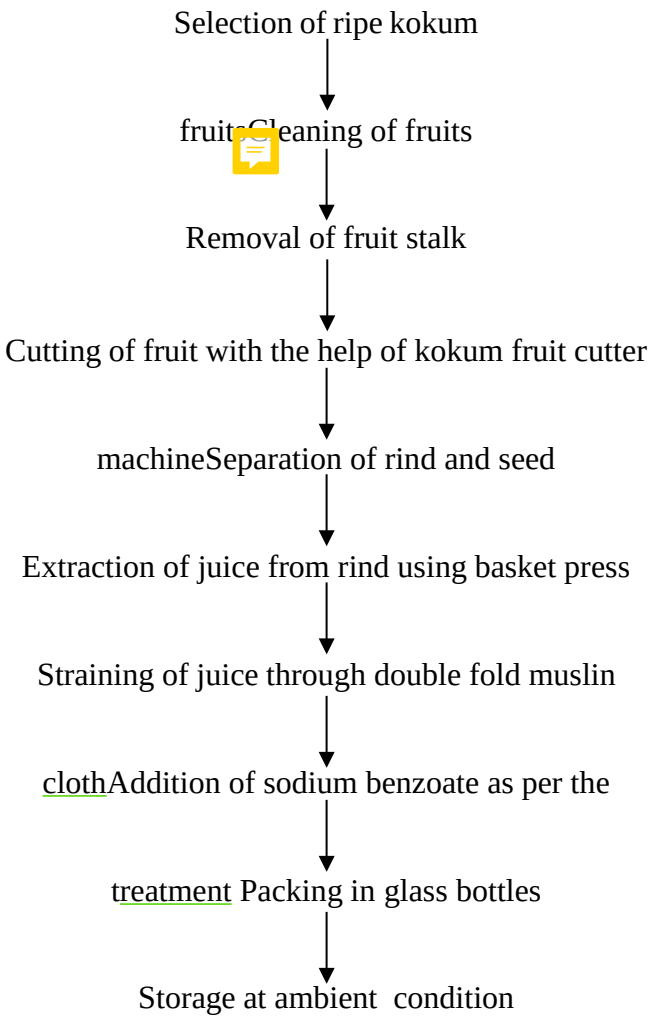
Dapoli. The fresh, ripe and mature fruits were selected and brought to laboratory for conducting the research.

1.1 Preparation of fruits and juice extraction

Fully ripe, fresh and sound kokum fruit were selected for extraction of kokum juice. The selected fruits were washed with water and sodium hypo-chloride to remove dirt and dust. The kokum fruits were then cut with help of kokum fruit cutter machine. Then the fruit rind and pulp with seed were separated. The kokum rind juice was extracted by using basket press. The extracted kokum juice was then strained through double fold muslin cloth. The sodium benzoate was then added in the kokum rind juice as per the treatment. Kokum juice was then filled in pre-sterilized glass bottles and the bottles were then sealed with threaded cap. The product, thus prepared was labeled and stored at ambient storage conditions for further investigations.

FLOW CHART - I

Preparation of kokum rind juice



1.2 Chemical parameter of kokum rind juice

1.2.1 Total Soluble Solids

The total soluble solids were determined by using Hand Refractometer (Atago Japan, 0-320B) and the values were corrected at 20 0C with the help of temperature correction chart (AOAC, 2020).

1.2.2 Titratable acidity

The titratable acidity was assessed by the standard method of Ranganna (1986). 10ml of sample was diluted with 100 ml distilled water. Further, 10ml of this aliquot was titrated against 0.1N NaOH solution to a pink end point using phenolphthalein as an indicator. The acidity as citric acid was calculated by the following expression:

$$\textbf{Titratable acidity}(\%) = \frac{Normality\ of\ alkali \times Titre\ reading \times Volume\ made \times Equivalent\ weight\ of\ acid}{Weight\ of\ sample\ taken \times Volume\ of\ sample\ taken\ for\ estimation \times 1000} \times 100$$

1.2.3 Reducing sugars

The reducing sugars were determined by the method of Lane and Eynon (1923) as described by Ranganna (1986) . A known weight of sample was taken in 250 ml volumetric flask. To this, hundred ml of distilled water was added and the contents were neutralized by 1 N sodium hydroxide. Then, 2 ml of 45 percent lead acetate was added to it. The contents were mixed well and kept for 10 minutes. Two ml of 22 percent potassium oxalate was added to it to precipitate the excess of lead. The volume was made to 250 ml with distilled water and solution was filtered through Whatman No. 4 filter paper. This filtrate was used for determination of reducing sugars by titrating it against the boiling mixture of Fehling ‘A’ and Fehling ‘B’ solutions (5 ml each) using methylene blue as indicator to a brick red end point. The results were expressed on per cent basis.

$$\textbf{Reducing sugars (\%)} = \frac{\text{Factor} \times \text{Dilution}}{\text{Titre reading} \times \text{Weight of sample}} \times 100$$

1.2.4 Total sugar

For inversion at room temperature, a 50 ml aliquot of clarified solution was transferred to 250 ml volumetric flask, to which, 10 ml of 50 percent HCl was added and then allowed to stand at room temperature for 24 hrs. It was then neutralized with 40 percent NaOH solution. The volume of neutralized aliquot was made to 250 ml with distilled water. This aliquot was used for determination of total sugars by titrating it against the boiling mixture of Fehling ‘A’ and Fehling ‘B’ (5ml each) using methylene blue as indicator to a brick red end point. The results were expressed on percent basis.

$$\textbf{Total sugars}\ (\%) = \frac{factor \times Dilution}{Titre\ reading \times weight\ of\ sample} \times 100$$

1.2.5 Anthocyanin

The anthocyanin content in juice was determined as per the procedure described by (Shriwastava and Kumar, 2015). Kokum juice was diluted with 0.1N HCl and allowed to equilibrate in the dark for 1hr. The (O.D) was recorded at 510 nm using spectrophotometer and total anthocyanin were estimated as given below,

$$\textbf{Total O.D. (Optical Density)/100ml} = \frac{\text{O. D. (Spectrophotometer reading) x volume made up x 100}}{\text{ml of juice taken}}$$

$$\textbf{Total Anthocynins (mg/100 ml)} = \frac{Total\ O.D \times\ 100ml}{87.3}$$

1.3 Microbial analysis

The microbial analysis of kokum fruit juice was carried out at 0 day and after 90 days of storage as per the method described by Kiiyukia (2003). Nutrient agar media was prepared by weighing required quality of nutrient agar. The media was autoclaved at 121°C for 20 min. then temperature of media was lowered to 40°C and used for plating.The plating was carried out with 1 ml. sample in sterile petri plate under the Laminar Air Flow. The sample of each treatment was taken on a separate petri plate, followed by pouring of approximately 20 ml. of media (35 to 40°C) on the sample and mixing was done by tilting plate properly, the plates were sealed with parafilm and incubated at 37°C for 24 hrs. To check the microbial count. Total microbial plate count was measured in colony forming unit/ml.

1.4 Sensory evaluation

The sensory quality of kokum rind juice was studied by preparing an RTS (6 per cent juice, 12°B TSS and 0.28 per cent acidity and remaining water) from various treatments. The sensory evaluation was done by using 9-point Hedonic scale. The tasting panel was consisting of 10 members. They were asked to evaluate the color, flavor and overall acceptability by a scoring rate on a 9 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 and 1=Dislike extremely. The different preferences as indicated by scores were evaluated by statistical methods

Result and discussion

Total soluble solids (°B)

The data presented in Table 1 shows that TSS content of kokum rind juice increase significantly in all treatment during storage period of 90 days.. The TSS content of kokum rind juice was significantly influenced by the treatments throughout the ambient storage period of 90 days. The maximum (12.92

°B) mean value of TSS was observed in the T1 (500 ppm) followed by the treatment, T2(12.69) and T3(12.49). The treatment T5 (1500 ppm) showed a minimum (12.23°Brix) mean value for the TSS content and the treatment T4 was at par with the treatment T5. As regards the storage, an increasing trend in the TSS content of kokum rind juice was notice 90 days of ambient storage condition. At the initial stage, the mean TSS was 12.5 ºBrix which was increased up to 12.94 ºBrix at 90 days ambient storage condition. The interaction effect between different treatments and ambient storage condition on the TSS of kokum rind juice was found to be statistically significant. After 90 days of ambient storage condition,the maximum (13.60 ºB) was recorded in the treatment T1.The minimum (12.47 ºB) TSS was observed in the treatment of T5 at ambient storage condition, however, it was at par treatment, except the T3 and T4. This condition could be due to the fact that the higher-level sodium benzoate reduced the biochemical change in kokum rind juice during storage. Investigation reveals that increase in TSS is probably due to the hydrolysis of polysaccharidesinto monosaccharides and the concentration of juice due to dehydration. The increase in TSS during storage was also reported by Shahnawaz et al. (2013) in orange juice and, Das (2009) in jamun processed products.

Titratable acidity (%)

It could be observed in Table 2 that different concentrations of sodium Benzoate showed a significant effect on the titratable acidity of kokum rind juice during ambient storage conditions. It was observed from that on an average highest mean acidity(3.80%) was observed in the treatment T1 (500 ppm) which was at par with the treatment T2(3.71). The lowest mean titratable acidity(3.53 %) was observed in the treatment T5 (1500 ppm) which was at par with the treatments T4 and T3.As regards the ambient storage period an increasing trend in titratable acidity content in kokum rind juice was noticed and the mean acidity significantly increased from 3.44 per cent at the initial stage to 3.84 per cent after 90 days of storage. The increase in titratable acidity might be due to the formation of organicacids by the degradation of ascorbic acid. An increase in acidity might be attributed due to the breakdown of pectin into pectinic acid or due to the formation of acid by the breakdown of polysaccharides or oxidation of reducing sugars. Similar findings of increasing acidity were observed by Hussain et al., (2011) in apple and apricot blend juice Desai (1991) in ber juice showed at ambient and low temperature, Bharwal and Shrera (2009)in hill lemon juice.

Reducing sugars(%)

It was clearly seen from the data that in Table 3 there was a significant difference among the treatments with respect to reducing sugars content in kokum rind juice. The treatment T1 (500 ppm) showed the significantly highest (9.05%) mean reducing sugar, followed by the treatmentT2. The minimum (8.40%) mean was observed in the treatment T5 which was at par with the treatments

T4 (8.47%) and T3 (8.55%).During ambient storage, the lowest reducing sugar content (8.26%) was rerecorded at 0 days.A significant increase in reducing sugar from 8.26 to 9.12 per cent was noticed up to 90 days of ambient storage condition. As regards, it is observed that in reducing sugar content increased with the increase in storage period in all the treatments. It could be attributed to the gradual inversion of non-reducing sugarsinto reducing sugars in an acidic medium. A similar result was reported by of Palaniswamy and Muthukrishnan (1974). who observed an increase in reducing sugar content in lemon juice during storage, Shrestha and Bhatia (1982) in aonla juice. Identical results were also reported by Bharwal and Shrera (2009) in hill lemon juice and Mhapralkar (2007) in jamun juice.. Mehmood et al. (2008) observed an increase in reducing the sugars of apple juice preserved with chemical preservatives and stored at ambient temperature for three months.

Total sugars (%)

It is evident from the data Table 4 that the different concentrations of sodium benzoate exhibited a significant effect on total sugars during storage at ambient conditions. The treatment T1(500) showed the significantly highest (10.09%) mean of total sugar followed by treatments T2 and T3. The lowest (9.58%) total sugar content was observed in the treatment T5 (1500 ppm) which was at par with treatment T4 (1250 ppm). The sodium benzoate level of either 1250 ppm or 1500 ppm didnot exhibit a significant increase in the total sugar content of kokum rind juice.As regards, ambient storage conditions, an increase in total sugar content was observed during90 days of ambient storage condition. The total sugars significantly increased from 9.46 per cent at the initial stage to 10.07 per cent up to 90 days of ambient storage condition.. The increase in total sugars might be due to the hydrolysis of polysaccharides like pectin, cellulose, starch, etc. and its conversion into simple sugars. The results obtained were similar to the findings of Bharwal and Shrera (2009) in hill lemon juice. Chobe (1991) in pomegranate juice and Patil and Jadhav (2001) in sweet orange juice.

Anthocyanins (mg/100g)

It could be observed from the Table 5 that there was a significant difference among the treatments with respect to anthocyanin content of kokum rind juice. The treatment T5 (1500 ppm) showed the highest (2.48 mg/100g) mean anthocyanin content which was at par with the treatment T4 . The minimum (1.85 mg/100g) mean was observed in the treatment T1. It is clear from the data presented data that the storage period had a significant effect on the anthocyanin content of kokum rind juice during 90 days of storage. At 0-day stage the anthocyanin, content was 2.52 mg/100g and it was decreased up to 1.92 mg/100g 90 days at storage period . After 90 days of storage period,

the maximum (2.44 mg/g) anthocyanin was observed in the treatment T5 which was at par with treatmentT4 . The minimum (1.21 mg/g) anthocyanin was observed on in the treatment T1 at 90 days storage. Anthocyanins were decreased during storage, this might be due to the hydrolysis of anthocyanins at higher temperature (Rhim2002) 81212097

Microbial analysis of kokum rind juice

Considering sodium benzoate concentrations, it was served that, the results were significant throughout the storage period with respect to yeast and bacterial count. From initial day of storage there was no growth of yeast and bacteria in treatment . At 3-month storage the lowest yeast and bacterial growth was found in treatment T5 which was significantly superior over rest of the treatments. While, highest yeast and bacterial load was recorded in T1 at 3-month storage, yeast and bacterial population was increased rapidly. From above findings it could be seen that, as the concentration of sodium benzoate increases, yeast and bacterial growth get decreased. Similar findings have been reported by, Oladipo et al. (2010) in some Nigerian fruit juices and Hussain et a/. (20 1 1) in apple and apricot blended juice. With respect to effect of storage conditions on the yeast and bacterial growth, it was observed that, as the storage period increases the yeast and bacterial load get increased at faster rate in ambient temperature (S,) as compared to cold storage (Sr). Similar findings have been reported by Emamifar (2010) in orange juice.

Sensory evaluation

The sensory score for changes in colour, flavour and overall acceptability of kokum rind juice during storage are presented in Table 7 to 9 . From the table it was observed that, mean score of colour, flavour and overall acceptability of kokum juice decreased with increased in storage period. Decrease in juice colour, flavour and overall acceptability with increased in storage period rnay be due to slight browning and increase of acids or may be due to effect of temperature on storage quality of juice, micro fermentation process at the end of storage. Similar findings have been reported by Khurdiya and Roy (1985), Bhandari (2004) in jamun juice, Shakoor et al., (2013) in strawberry juice

Table 1: Effect of sodium benzoate concentrations on Total Soluble Solid (°B) of kokum rind juice under ambient storage conditions.

Treatment	TSS (°B)				
	Storage period (Days)				
	0	30	60	90	Mean
T1	12.03	12.67	13.40	13.60	12.92
T2	12.04	12.63	12.87	13.23	12.69
T3	12.04	12.47	12.67	12.77	12.49
T4	12.06	12.40	12.37	12.63	12.36
T5	12.07	12.13	12.23	12.47	12.23
Mean	12.05	12.46	12.71	12.94	
		S.Em ±		CD at 5%	
Treatment (T)		0.06		0.16	
Storage (S)		0.05		0.14	
Interaction (TxS)		0.11		0.32	

Table 2: Effect of sodium benzoate concentrations on titratable acidity (%) of Kokum rind juice under ambient storage conditions

Treatment	Titratable acidity (%)				
	Storage period (Days)				
	0	30	60	90	Mean
T1	3.43	3.74	3.92	4.12	3.80
T2	3.45	3.66	3.79	3.94	3.71
T3	3.44	3.55	3.73	3.80	3.63
T4	3.44	3.52	3.63	3.69	3.57
T5	3.45	3.46	3.57	3.63	3.53
Mean	3.44	3.59	3.73	3.84	
		S.Em ±		CD at 5%	
Treatment (T)		0.04		0.11	
Storage (S)		0.03		0.09	
Interaction (TxS)		0.07		NS	

Table 3: Effect of sodium benzoate concentrations on reducing sugars (%) of kokum rindjuice under ambient storage conditions

Treatment	Reducing sugars (%)				
	Storage period (Days)				
	0	30	60	90	Mean
T1	8.24	8.62	9.45	9.86	9.05
T2	8.26	8.49	9.02	9.39	8.79
T3	8.26	8.38	8.68	8.89	8.55
T4	8.27	8.30	8.52	8.80	8.47
T5	8.27	8.28	8.39	8.64	8.40
Mean	8.26	8.42	8.81	9.12	
		S.Em ±		CD at 5%	
Treatment(T)		0.09		0.24	
Storage(S)		0.08		0.22	
Interaction (TxS)		0.16		NS	

Table 4: Effect of sodium benzoate concentrations on total sugar (%) content of kokum rindjuice under ambient storage conditions

Treatment	Total sugar (%)				
	Storage period (Days)				
	0	30	60	90	Mean
T1	9.45	10.00	10.29	10.62	10.09
T2	9.46	9.88	10.02	10.22	9.89
T3	9.46	9.68	9.78	9.91	9.71
T4	9.47	9.56	9.69	9.85	9.64
T5	9.47	9.52	9.62	9.73	9.58
Mean	9.46	9.73	9.88	10.07	
		S.Em ±		CD at 5%	
Treatment(T)		0.058		0.17	
Storage(S)		0.052		0.15	
Interaction (TxS)		0.11		NS	

Table 5: Effect of sodium benzoate concentrations on anthocyanins (mg/100g) of kokum rind juice under ambient storage conditions

Treatment	Anthocyanins (mg/100g)				
	Storage period (Days)				
	0	**30**	**60**	**90**	**Mean**
T1	2.53	2.02	1.63	1.21	1.85
T2	2.54	2.24	1.99	1.55	2.08
T3	2.51	2.39	2.29	2.08	2.32
T4	2.51	2.44	2.44	2.33	2.43
T5	2.53	2.46	2.48	2.44	2.48
Mean	2.52	2.31	2.17	1.92	
		S.Em ±		**CD at 5%**	
Treatment(T)		0.05		0.15	
Storage(S)		0.05		0.13	
Interaction (TxS)		**0.10**		**0.29**	

Table 6: Effect of sodium benzoate concentrations on microbial count (cfu/ml) of Kokum rind juice under ambient storage conditions

Microbial count (No. × 10^3 cfu/ml)		
Treatment	**Storage period (Days)**	
	0	**90**
T1	ND	3.34
T2	ND	2.91
T3	ND	2.68
T4	ND	2.37
T5	ND	1.76
Mean	**ND**	**2.61**
S.Em ±	**0**	**0.03**
CD at 5%	**0**	**0.07**

Table 7: Effect of sodium benzoate concentrations on sensory score for colour of kokum rind juice stored in ambient conditions

Treatment	Sensory score for colour				
	Storage period (Days)				
	0	30	60	90	Mean
T1	7.24	5.70	5.70	5.30	5.99
T2	7.30	6.20	5.80	5.30	6.15
T3	7.60	6.20	6.14	5.40	6.34
T4	7.60	6.10	5.90	5.70	6.33
T5	7.70	6.30	6.10	5.90	6.50
Mean	7.49	6.10	5.93	5.52	
		S.Em ±		CD at 5%	
Treatment(T)		0.06		0.16	
Storage(S)		0.05		0.14	
Interaction (TxS)		0.11		NS	

Table 8: Effect of sodium benzoate concentrations on sensory score for flavour of kokumrind juice under ambient storage conditions

Treatment	Sensory score for flavour				
	Storage period (Days)				
	0	30	60	90	Mean
T1	7.40	6.50	6.17	5.80	6.47
T2	7.40	6.90	6.46	5.79	6.64
T3	7.60	7.24	6.60	6.30	6.94
T4	7.70	7.10	6.60	6.29	6.93
T5	7.80	7.40	6.69	6.59	7.12
Mean	7.58	7.03	6.50	6.15	6.82
		S.Em ±		CD at 5%	
Treatment(T)		0.10		0.29	
Storage(S)		0.09		0.26	
Interaction (TxS)		0.19		NS	

Table 9: Effect of sodium benzoate concentrations on overall acceptability of kokum rindjuice under ambient storage conditions

Treatment	Sensory score for overall acceptability				
	Storage period (Days)				
	0	**30**	**60**	**90**	**Mean**
T1	7.11	5.95	6.14	5.55	6.19
T2	7.35	6.25	6.35	5.65	6.40
T3	7.45	6.30	6.35	5.57	6.42
T4	7.65	6.30	6.60	6.00	6.64
T5	7.72	6.50	6.76	6.25	6.81
Mean	7.46	6.26	6.44	5.81	
		S.Em ±		**CD at 5%**	
Treatment(T)		0.03		0.10	
Storage(S)		0.03		0.09	
Interaction (TxS)		**0.07**		**NS**	

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