

Final Data Report 2013-2014

Objective 4



A data report submitted to the National Mango Board
Aug 15, 2015

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Overview

A) Cell culture Study

Breast Cancer: Breast cancer is a major cause of premature death in women. Indeed, it is estimated that in the U.S. more than 230,000 additional cases of breast cancer will be diagnosed this year. Whereas most early stage breast cancers are estrogen-receptor (ER)-positive and respond to endocrine therapy, later stage breast cancers tend to be ER-negative and aggressive, requiring treatment with cytotoxic drugs. Inflammation has been identified as a significant contributor to the initiation and progression of breast cancer.

Cell lines vs. non-modified tumor cells: Much of the in vitro (cell culture research) is being performed in immortalized cell lines that do not display all the characteristics that primary cells have. For this research, primary tumor cells were used that were not genetically modified from the original characteristics displayed in the tumor.

Objective of this study: In order to more accurately quantify the polyphenol-content of

mangos as it relates to their health-benefits (inflammation), we performed an efficacy-guided fractionation of mango polyphenols of 5 different varieties where we fractionate mango polyphenols into smaller classes and groups according to their anti-inflammatory efficacy.

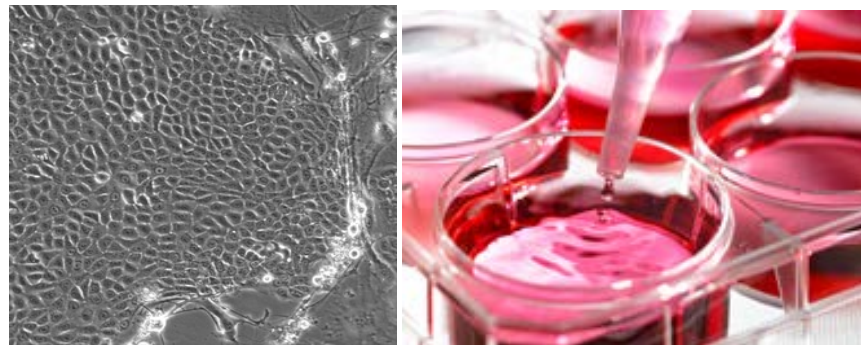
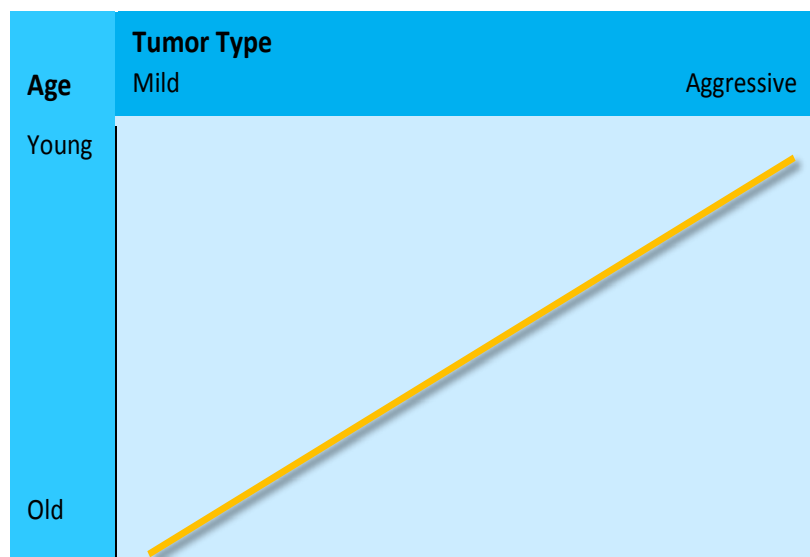


Figure 1: a) tumor cells growing on a cell culture dish (microscopy), b) cell culture plate

Major Findings:

Results show that even low concentrations of mango polyphenols caused cell death in cancer cells (but not in non-cancer, normal cells). The more aggressive (more late stage) the tumor cells, the more sensitive these appeared to be to the treatment with mango polyphenols (**Figures 2-6**). Overall, mango was more effective the younger the woman and the more aggressive the tumor type.



Anti-Cancer Activity of Mango Polyphenols

The sample size (n=6) was not sufficient to determine this correlation with statistical certainty, but this hypothesis should be used as covariate in any future breast cancer trial with human clinical subjects.

Benefits to the Mango Industry:

Data indicate that mango polyphenols have a high anti-cancer efficacy in tumor cells that were not genetically modified in vitro. This indicates that mango polyphenols may have a high efficacy in a future human clinical trial with breast cancer patients.

These findings will be used in combination with the survey questionnaire for breast cancer patients that is described in part C).

These results will be used in the manuscript for part B).

Results:

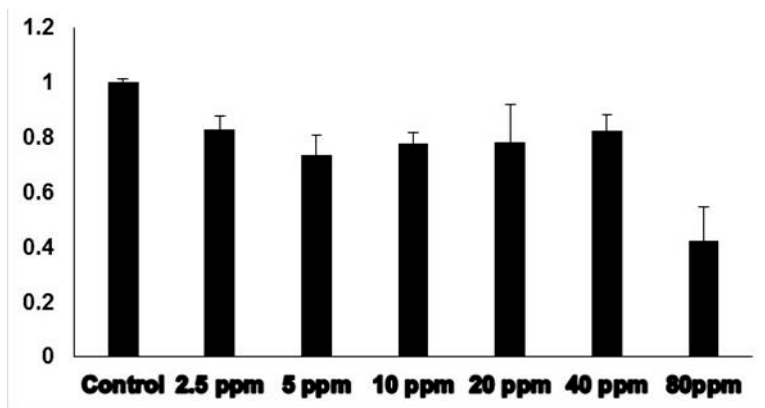


Figure 2: Female, 74 years old with grade II adenocarcinoma, IC50= 65ppm

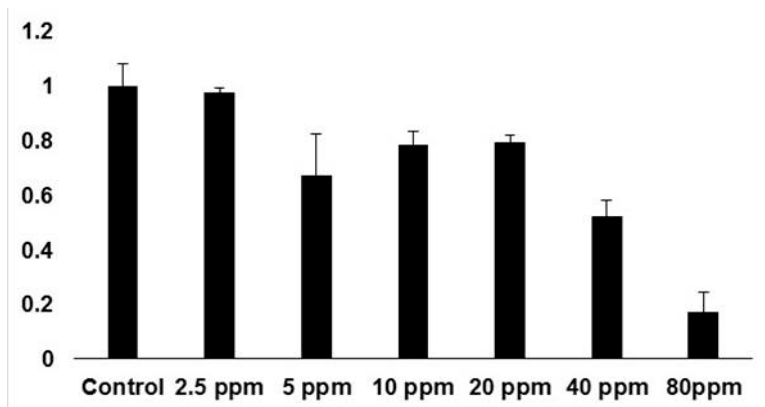


Figure 3: Female, 51 years old with adenocarcinoma, IC50= 40ppm

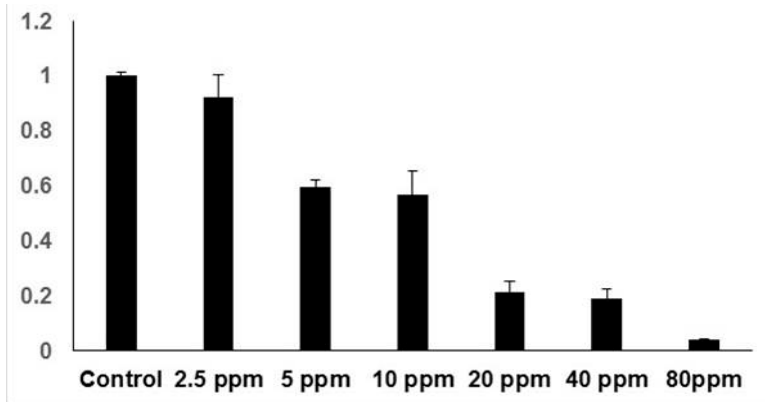


Figure 4: Female, 50 years adult TNM stage IIB, grade 3, primary ductal carcinoma, IC50=10ppm

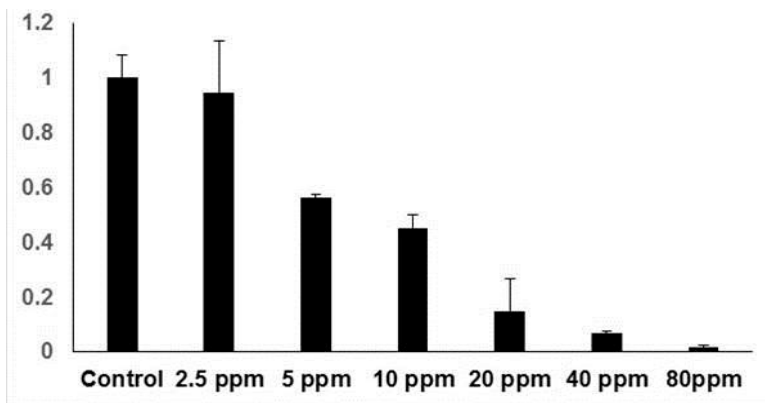


Figure 5: Female, 41 years old, TNM stage IIA, grade 3, primary ductal carcinoma, C50= 5ppm

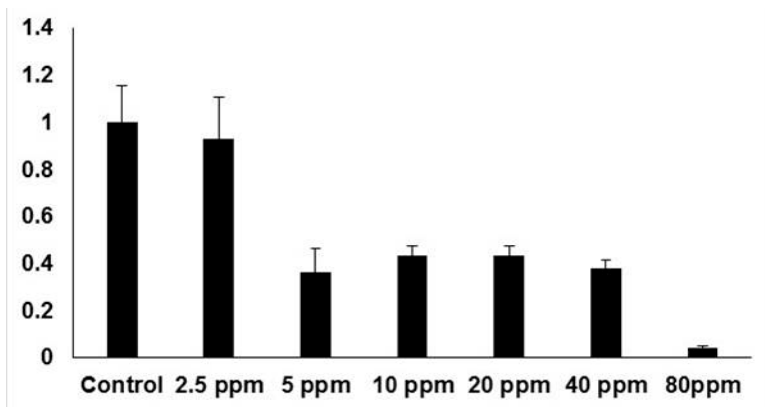


Figure 6: Female, 23 years old with TNM stage IIB, grade 3, primary ductal carcinoma IC50 4 ppm

B) Animal study with mice with implanted tumors

This partial objective presents a change to the originally proposed research. Currently our fiscal office is processing a formal request for this change to the NMB, so that we can use funding from objective 4 for this partial project.

Based on recommendations from a collaborating oncologist, highly aggressive tumor cells were implanted into mice. Mice were treated with mango polyphenols, pyrogallate (a microbial metabolite from the colon) or a common cancer drug (5FU). The animal portion of this study is just being completed (Aug 1, 2014). We have collected tissues and will now begin the molecular analysis. Preliminary results indicate that mango and pyrogallate are highly effective in shrinking the tumor size in treated animals (**Figure 7**).

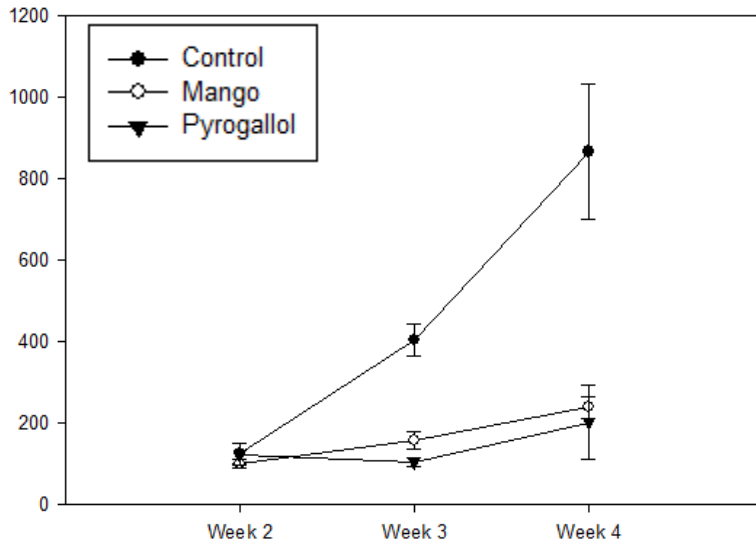


Figure 7. Final tumor volumes at week 4 with 0.8mg/kg/day mango extract and 0.2mg/kg/day pyrogallol (the major microbial metabolite of mango polyphenols)

Biomarkers for Inflammation were significantly reduced (Figure 8)

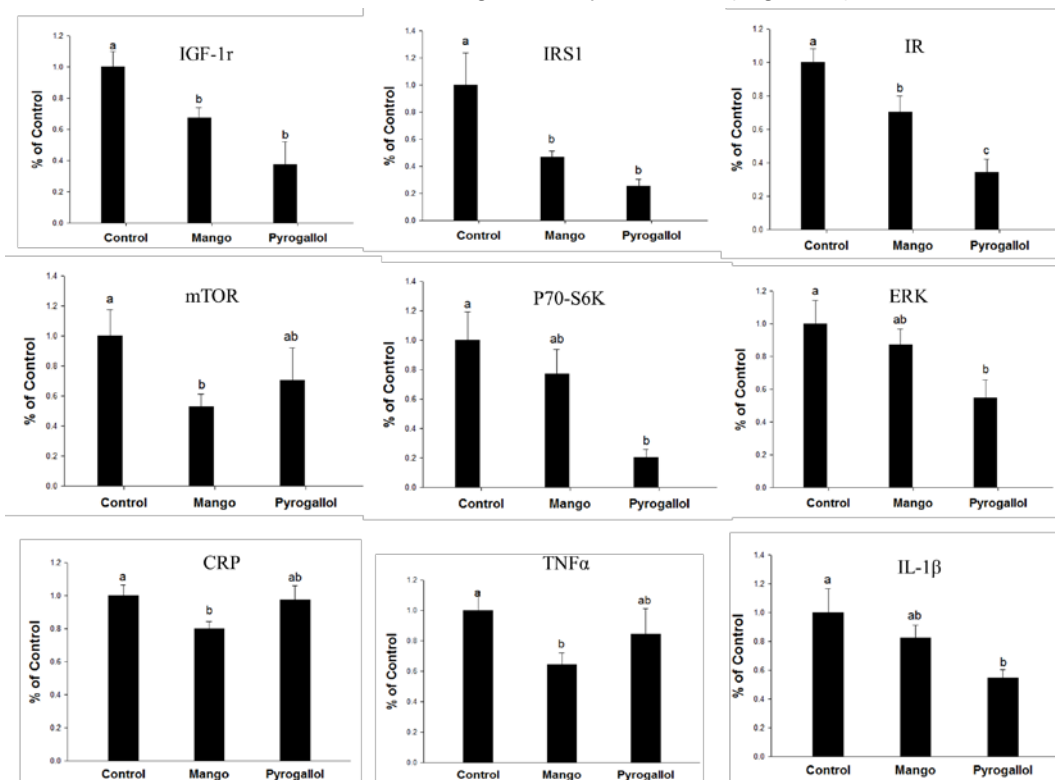


Figure 8A. Phosphorylated protein data for controls, 0.8mg/kg/day mango extract and 0.2mg/kg/day pyrogallol in mice with breast cancer xenografts. The first six graphs represent critical proteins along the mTOR pathway involved in proliferation and inflammation and the bottom row presents inflammation biomarkers.

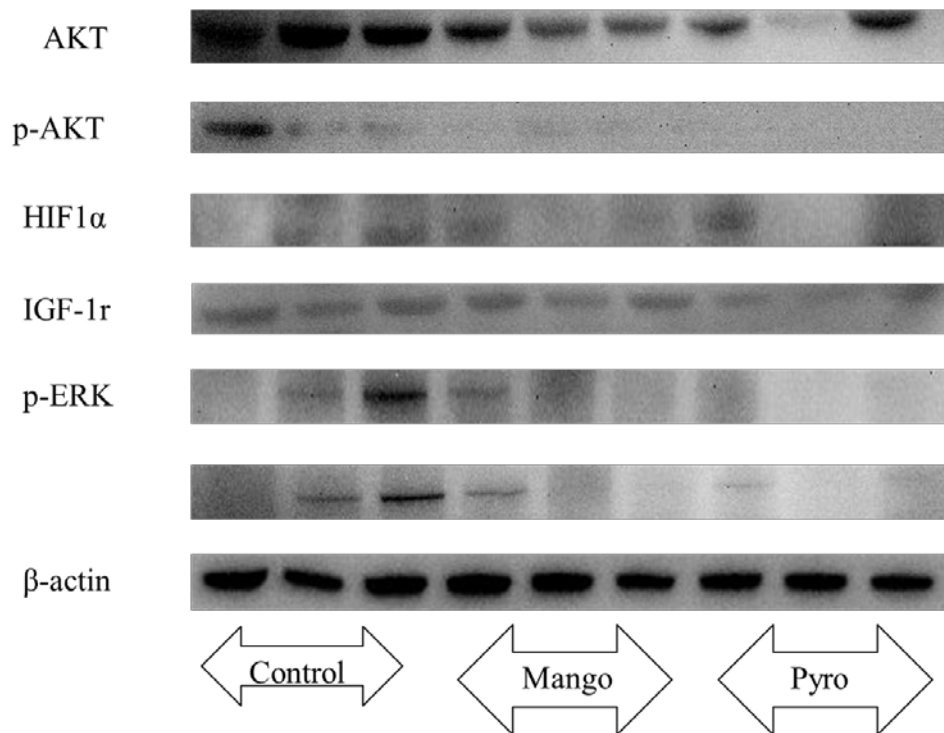


Figure 8B. Western blot results from tumors following 4 week *in vivo* oral treatment with 0.8mg/kg/day mango extract or 0.2mg/kg/day pyrogallol.

Additionally, the molecular mechanisms underlying the reduction of tumor size is based on the reduction of proliferation in cancer cells that for a major part are based on polyphenol interactions with AKT, and AMPK a central molecule in inflammation and cell growth. The figure below is demonstrating some of the proposed mechanisms (Figure 9).

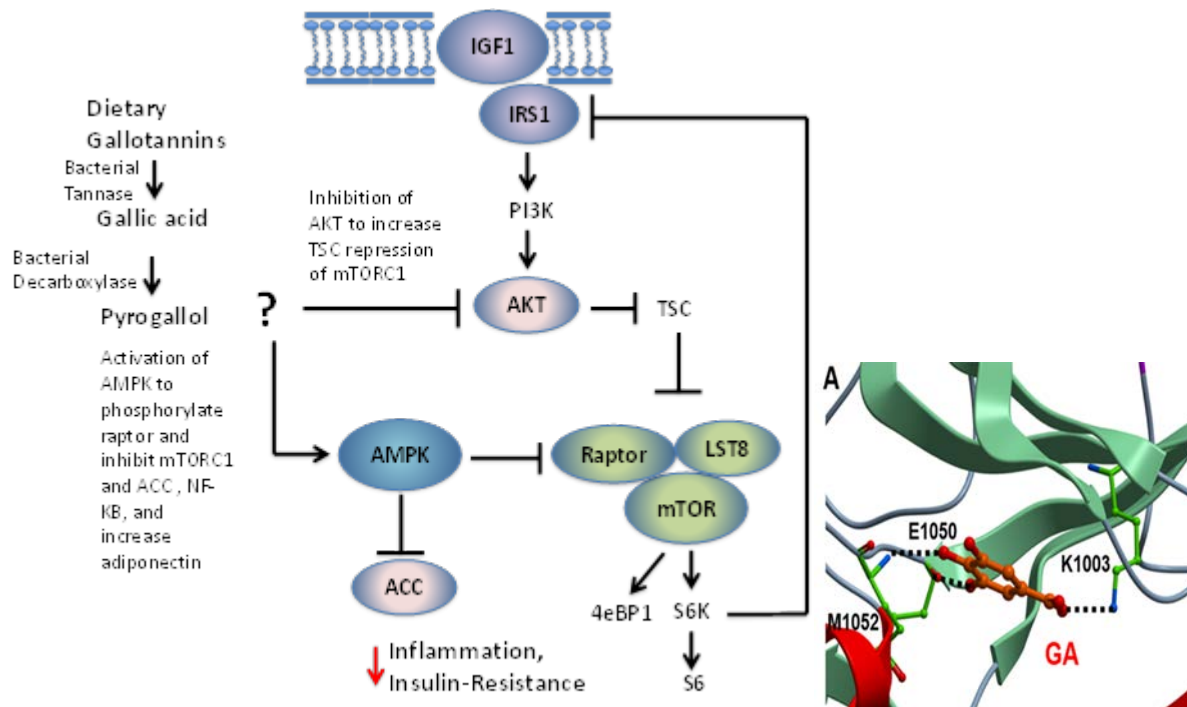


Figure 9: Overview of proposed mechanism

Figure 10: In situ modeling of pyrogallol with AMPK

It seems that pyrogallol specifically interacts with AMPK, a crucial regulator of inflammation and proliferation (**Figure 10**).

Major Findings: This preliminary data indicates that mango and the microbial metabolite from the colon (pyrogallate) are highly effective in reducing tumor size in animals after just 4 weeks of daily treatment. Since pyrogallate is present in our body after the consumption of mango, there may be a protection against cancer not only from the primary mango compounds but also from their metabolites such as pyrogallate. Molecular analysis of these animal samples has been completed and confirmed with in vitro experiments.

Benefits to the Mango Industry: This animal study demonstrates that not only primary mango compounds but also one of their metabolites are highly bioactive.

One of the major critique for polyphenols in general is that the absorption is too low for them to be bioactive within a body. This study demonstrates that pyrogallate a metabolite that is absorbable is highly bioactive against a very aggressive type of breast cancer.

We are planning a scientific publication, a press release and follow grant proposals to federal agencies using these data.

C) Survey for women with breast cancer

This survey is designed to determine the current disease status of participating women and collect information regarding their interest in participating in a human clinical trial for breast cancer patients.

In order to design a follow up study in patients with breast cancer it is crucial to determine the willingness of breast cancer patients to undergo different procedures, consume the study treatment etc.

The survey is publically available but has been directed to target Breast Cancer Support group within our county (Brazos County) and the University campus community.



AgriLIFE RESEARCH & EXTENSION
Texas A&M System

FIGHT against Breast Cancer

ATM | TEXAS A&M UNIVERSITY

Prevention of Breast Cancer With Mango Fruits?

Natural compounds in fruits and vegetables have been found to have cancer-preventive properties. Researchers at Texas A&M University are conducting a short online survey: If you are a

Breast Cancer Patient or Survivor

please participate; this will help us to design optimal future breast cancer prevention studies in the area of nutrition.

This survey is anonymous, will take you about 7-10 min, and you may participate in a raffle for \$50-prizes upon completion.

For more information please check this link
https://agrilife.az1.qualtrics.com/SE/?SID=SV_emoAC1lcU7WCYYJ or
email us: breastcancerstudy14@gmail.com



Benefits to the Mango Industry: Major benefits include that knowledge regarding the study preferences of breast cancer patients will help us to design a much more targeted study that will be more likely to succeed. Additionally, participants may leave their contact information so that we already can recruit a patient pool for a future human clinical trial.

This survey is now completed and over 200 women participated. Overall, women with breast cancer are eager to explore dietary intervention studies in the prevention or even treatment of breast cancer. A large percentage of women would be in favor of participating an intervention study with mango. The duration of the study was not an important factor, however, most women would prefer minimally invasive procedures, e.g. collection of blood urine or feces, in contrast to needle-biopsies of breast tissue.

Expected Completion: This survey is completed and we are preparing the manuscript.

Results:

Q1. Are you male or female?

Q1	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Female	109	87.2	109	87.2
Male	16	12.8	125	100

Q2. What is your age range?

Q2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
18-25	45	36	45	36
25-35	15	12	60	48
35-45	6	4.8	66	52.8
45-55	27	21.6	93	74.4
55-65	25	20	118	94.4
65+	7	5.6	125	100

Q3. Do you currently have breast cancer?

Q3	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	118	94.4	118	94.4
Yes	7	5.6	125	100

Q4. Have you recently recovered from breast cancer or are you in remission?

Q4	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	73	58.4	73	58.4
Yes	52	41.6	125	100

Q5. What type of breast cancer do/did you have?

Q5	Frequency	Percent	Cumulative Frequency	Cumulative Percent
ER+	28	57.14	28	57.14
ER-	21	42.86	49	100
Frequency Missing	76			

Q7. Have you undergone chemotherapy?

Q7	Frequency	Percent	Cumulative Frequency	Cumulative Percent
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No	67	72.04	67	72.04
Yes	26	27.96	93	100
Frequency Missing	32			

Q8. If yes, for how long?

Q8	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 year	3	10.34	3	10.34
1-2 years	1	3.45	4	13.79
3 years	1	3.45	5	17.24
3+ years	1	3.45	6	20.69
6 months	16	55.17	22	75.86
< 3 months	7	24.14	29	100
Frequency Missing	32			

Q9. Have you had any surgery related to your breast cancer?

Q9	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	36	39.56	36	39.56
Yes	55	60.44	91	100
Frequency Missing	34			

Q10. Which type of surgery?

Q10	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Double Mastectomy	8	14.55	8	14.55
Removal of Tumor Tissue	36	65.45	44	80
Single Mastectomy	11	20	55	100
Frequency Missing	70			

Q21. Do you currently take any vitamin pills, botanical supplements?

Q21	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	43	45.26	43	45.26
Yes	52	54.74	95	100
Frequency Missing	30			

Q23. How often do you take botanical supplements?

Q23	Frequency	Percent	Cumulative Frequency	Cumulative Percent
2-3 Times a Month	2	2.15	2	2.15

2-3 Times a Week	6	6.45	8	8.6
Daily	23	24.73	31	33.33
Less than Once a Month	3	3.23	34	36.56
Never	58	62.37	92	98.92
Once a Month	1	1.08	93	100

Q11. Please indicate your answer by selecting on the point scale:

1 Are you interested in nutritional prevention of breast cancer or recurring breast cancer?

Q11-1	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 Completely Disagree	1	1.18	1	1.18
2 Strongly Disagree	3	3.53	4	4.71
3 Mostly Disagree	3	3.53	7	8.24
4 Neither Agree nor Disagree	5	5.88	12	14.12
5 Mostly Agree	8	9.41	20	23.53
6 Agree	30	35.29	50	58.82
7 Strongly Agree	35	41.18	85	100

2 Do you think that botanical supplements would be beneficial in preventing breast cancer?

Q11-2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 Completely Disagree				
2 Strongly Disagree	1	1.16	1	1.16
3 Mostly Disagree	6	6.98	7	8.14
4 Neither Agree nor Disagree	24	27.91	31	36.05
5 Mostly Agree	24	27.91	55	63.95
6 Agree	17	19.77	72	83.72
7 Strongly Agree	14	16.28	86	100

3 Do you think that a healthy nutrition rich in fruits and vegetables may be beneficial in preventing breast cancer or recurring breast cancer?

Q11-3	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 Completely Disagree	1	1.18	1	1.18
2 Strongly Disagree	1	1.18	2	2.35
3 Mostly Disagree	6	6.98	7	8.14
4 Neither Agree nor Disagree	8	9.41	10	11.76
5 Mostly Agree	15	17.65	25	29.41
6 Agree	31	36.47	56	65.88
7 Strongly Agree	29	34.12	85	100

4 Do you think that obesity or overweight contribute to getting breast cancer?

Q11-4	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 Completely Disagree	1	1.18	1	1.18
2 Strongly Disagree	4	4.71	5	5.88
3 Mostly Disagree	5	5.88	10	11.76
4 Neither Agree nor Disagree	15	17.65	25	29.41
5 Mostly Agree	15	17.65	40	47.06
6 Agree	21	24.71	61	71.76
7 Strongly Agree	24	28.24	85	100

5 Do you think that stress contributes to getting breast cancer?

Q11-5	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 Completely Disagree	2	2.35	2	2.35
2 Strongly Disagree	4	4.71	6	7.06
3 Mostly Disagree	7	8.24	13	15.29
4 Neither Agree nor Disagree	13	15.29	26	30.59
5 Mostly Agree	14	16.47	40	47.06
6 Agree	21	24.71	61	71.76
7 Strongly Agree	24	28.24	85	100

6 Do you think that physical inactivity contribute to getting breast cancer?

Q11-6	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 Completely Disagree	1	1.18	1	1.18
2 Strongly Disagree	5	5.88	6	7.06
3 Mostly Disagree	5	5.88	11	12.94
4 Neither Agree nor Disagree	22	25.88	33	38.82
5 Mostly Agree	12	14.12	45	52.94
6 Agree	17	20	62	72.94
7 Strongly Agree	23	27.06	85	100

7 Did you change your nutrition after being diagnosed with breast cancer to a more healthy diet?

Q11-7	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 Completely Disagree	12	14.29	12	14.29
2 Strongly Disagree	2	2.38	14	16.67
3 Mostly Disagree	9	10.71	23	27.38
4 Neither Agree nor Disagree	17	20.24	40	47.62

5 Mostly Agree	17	20.24	57	67.86
6 Agree	14	16.67	71	84.52
7 Strongly Agree	13	15.48	84	100

8 Did you increase your intake of fruits and vegetables after being diagnosed with breast cancer?

Q11-8	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 Completely Disagree	10	11.9	10	11.9
2 Strongly Disagree	7	8.33	17	20.24
3 Mostly Disagree	6	7.14	23	27.38
4 Neither Agree nor Disagree	21	25	44	52.38
5 Mostly Agree	12	14.29	56	66.67
6 Agree	14	16.67	70	83.33
7 Strongly Agree	14	16.67	84	100

9 Would you be interested in participating in nutritional studies that investigate the effects of fruits, vegetables or dietary supplements on the risk for recurring breast cancer?

Q11-9	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 Completely Disagree	8	9.3	8	9.3
2 Strongly Disagree	7	8.14	15	17.44
3 Mostly Disagree	3	3.49	18	20.93
4 Neither Agree nor Disagree	17	19.77	35	40.7
5 Mostly Agree	11	12.79	46	53.49
6 Agree	20	23.26	66	76.74
7 Strongly Agree	20	23.26	86	100

Q14. Which of the following fruits or vegetables do you think would be beneficial in improving health and possibly preventing the recurrence of breast cancer?

The FREQ Procedure

Q14-1	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	90	72	90	72
Acai	35	28	125	100

Q14-2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	116	92.8	116	92.8
Acerola	9	7.2	125	100

Q14-3	Frequency	Percent	Cumulative	Cumulative
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			Frequency	Percent
	63	50.4	63	50.4
Blueberries	62	49.6	125	100

Q14-4	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	92	73.6	92	73.6
Cherries	33	26.4	125	100

Q14-5	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	87	69.6	87	69.6
Carrots	38	30.4	125	100

Q14-	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	109	87.2	109	87.2
Curcumin	16	12.8	125	100

Q14-7	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	78	62.4	78	62.4
Green Tea	47	37.6	125	100

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The FREQ Procedure

Q14-8	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	82	65.6	82	65.6
Mango	43	34.4	125	100

Q14-9	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	102	81.6	102	81.6
Onion	23	18.4	125	100

Q24. Please rank (grab and drag) the following fruits in the order which you believe these might be effective in preventing breast cancer (from most to least effective).

1 Banana

Q24-1	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1	3	4.05	3	4.05
2	16	21.62	19	25.68
3	9	12.16	28	37.84
4	13	17.57	41	55.41
5	7	9.46	48	64.86
6	12	16.22	60	81.08
7	14	18.92	74	100
Frequency Missing	51			

2 Mango

Q24-2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1	15	20.27	15	20.27
2	9	12.16	24	32.43
3	13	17.57	37	50
4	13	17.57	50	67.57
5	14	18.92	64	86.49
6	9	12.16	73	98.65
7	1	1.35	74	100
Frequency Missing	51			

3 Pomegranate

Q24-3	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1	13	17.57	13	17.57
2	18	24.32	31	41.89
3	12	16.22	43	58.11
4	16	21.62	59	79.73
5	6	8.11	65	87.84
6	5	6.76	70	94.59
7	4	5.41	74	100
Frequency Missing	51			

4 Grape

Q24-4	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1	2	2.7	2	2.7
2	6	8.11	8	10.81

3	6	8.11	14	18.92
4	10	13.51	24	32.43
5	21	28.38	45	60.81
6	19	25.68	64	86.49
7	10	13.51	74	100
Frequency Missing	51			

5 Nuts

Q24-5	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1	2	2.7	2	2.7
2	8	10.81	10	13.51
3	8	10.81	18	24.32
4	10	13.51	28	37.84
5	15	20.27	43	58.11
6	12	16.22	55	74.32
7	19	25.68	74	100
Frequency Missing	51			

6 Blueberries

Q24-6	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1	28	37.84	28	37.84
2	8	10.81	36	48.65
3	15	20.27	51	68.92
4	6	8.11	57	77.03
5	5	6.76	62	83.78
6	10	13.51	72	97.3
7	2	2.7	74	100
Frequency Missing	51			

7 Acai

Q24-7	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1	11	14.86	11	14.86
2	9	12.16	20	27.03
3	11	14.86	31	41.89
4	6	8.11	37	50
5	6	8.11	43	58.11
6	7	9.46	50	67.57
7	24	32.43	74	100
Frequency Missing	51			

Q13. Which of the following fruits or vegetables would you be interested in participating in a research study with (consuming 1-2 servings of that fruit/vegetable per day for several weeks - of course, these would be prepared in the most delicious way :)).

Q13-	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	98	78.4	98	78.4
Acai	27	21.6	125	100

Q13-2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	114	91.2	114	91.2
Acerola	11	8.8	125	100

Q13-3	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	80	64	64	80
Blueberries	45	36	125	100

Q13-4	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	81	64.8	81	64.8
Cherries	44	35.2	125	100

Q13-5	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	85	68	85	68
Carrots	40	32	125	100

Q13-6	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	111	88.8	111	88.8
Curcumin	14	11.2	125	100

Q13-7	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	84	67.2	84	67.2
Green Tea	41	32.8	125	100

Q13-8	Frequency	Percent	Cumulative Frequency	Cumulative Percent
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	82	65.6	82	65.6
Mango	43	34.4	125	100

Q13-9	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	105	84	105	84
Onion	20	16	125	100

Q15. If you decided to participate in a nutritional research study, how long should it maximally take?

Q15	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1 week	6	8.82	6	8.82
12 weeks	10	14.71	16	23.53
16 weeks	1	1.47	17	25
2 weeks	7	10.29	24	35.29
4 weeks	11	16.18	35	51.47
8 weeks	12	17.65	47	69.12
I don't care	21	30.88	68	100
Frequency Missing	57			

Q16. Would you tolerate 1-2 of any of the following procedures for a nutritional research study?
(check all that you would tolerate)
The FREQ Procedure

Q16-1	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	70	56	70	56
Blood collection	55	44	125	100

Q16-2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	67	53.6	67	53.6
Urine collection	58	46.4	125	100

Q16-3	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	97	77.6	97	77.6
Stool collection	28	22.4	125	100

Q16-4	Frequency	Percent	Cumulative Frequency	Cumulative Percent
	104	83.2	104	83.2

Needle biopsy of breast tissue (not a large core biopsy)	21	16.8	125	100
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Summary

The study cohort consists of 125 people, 87% of them are women; 36% are young adults aged 18-25 years old, and 26% are older than 55. Most of them (94%) do not currently have breast cancer, and 42% recently recovered from breast cancer or in remission.

Of the 49 people who provided information, 57% have ER+ type of cancer, and the rest have ER- breast cancer. Of the 93 people who provided information, 28% have undergone chemotherapy, and 60% (n=55) had surgery related to breast cancer (sample size N=91). Of the 55 people who had surgery, 20% had single mastectomy, 15% had double mastectomy, and the rest had tumor tissues removed.

Of the 95 people who provided information, 55% currently take vitamin pills, or botanical supplements; 25% take them daily, and 6% take them 2-3 times a week, and the remaining 24% people take them less frequently. Of the 85 people who provided information, the following agree or strongly agree with these statements—76% are interested in nutritional prevention of breast cancer or recurring breast cancer, 36% think that botanical supplements would be beneficial in preventing breast cancer, 71% think that a healthy nutrition rich in fruits and vegetables may be beneficial in preventing breast cancer or recurring breast cancer, 53% think that obesity or overweight contribute to getting breast cancer, 53% think that stress contributes to getting breast cancer, 47% think that physical inactivity contribute to getting breast cancer, 32% changed nutrition after being diagnosed with breast cancer to a more healthy diet, 33% increased intake of fruits and vegetables after being diagnosed with breast cancer, 47% were interested in participating in nutritional studies that investigate the effects of fruits, vegetables or dietary supplements on the risk for recurring breast cancer.

When asked which fruits or vegetables they think would be beneficial in improving health, blueberries received the most votes, from 50% of the surveyed group. It is followed by green tea, chosen by 38% of the sample, and then mango, chosen by 34% of women and this indicates a significant interest in mangos and their effects in breast cancer prevention.

The participants were also asked to rank different fruits in the order which they believe these might be effective in preventing breast cancer, and 74 people provided this information. Among them, 38% ranked blueberries as the most effective, followed by mango, from 20% of the sample; and pomegranate, 18%.

When asked for which of the following fruits or vegetables they would be interested in participating in a research study, 36% of the survey sample chose blueberries, 35% chose cherries, 34% chose mangos, 33% chose green tea.

For the question on how long the future study should last, 68 people responded, and 35% prefer the study to be shorter than or equal to 4 weeks but the same percentage of individuals who did not care about the length of the study. The participants were also asked whether they would tolerate one or two of the following procedures for a nutritional research study. 44% can tolerate blood collection, 46% urine collection, 22% stool collection, 17% needle biopsy of breast tissue.

Conclusion: Overall, it seems that a clinical pilot study investigating the role of mango consumption in the prevention of breast cancer would be very feasible, where many subjects would not care how long it would take – this would make a 4-12 week study very feasible. Only 17% would tolerate the collection of breast tissue using a needle biopsy (like during blood collection). This indicates that the recruitment of subjects will be a significant factor in completion of a breast cancer study where breast tissue would need to be collected. It has to be

considered that these subjects were not recruited in a hospital setting where a higher tolerance to needle biopsies could be expected.

For a study that would use blood and urine collection, no issues with recruitment would be expected. Overall, this survey gives a very useful summary of preferences of human subjects with breast cancer for future clinical intervention studies with mangos.

It is recommended to complete the detailed pharmacokinetics studies before starting a breast cancer human clinical trial in order to match subjects by their ability to metabolize mango polyphenolics and generate bioactive metabolites.