Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

ORIGINAL RESEARCH ARTICLE

Pharmaceutico-Analytical Study and Standardisation of *Ashtanga Ghrita*

Author: Yashika Singh[1]

Co Authors: D N S Gautam[2]

[1,2]Department of Rasa Shastra and Bhaishjya Kalpana, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, UP, India

ABSTRACT

*Sneha Kalpana*s are an integral part of *Ayurvedic* pharmaceutics, of which, *Ghrita Kalpanas* are widely used for external as well as internal administration in the form of *Paana, Nasya, Basti, Abhyanga*. *Ashtanaga Ghrita* is explained in *Ashtanga Hrudya* for its use in enhancement of *Medha*, *Smriti*, *Vaka* and *Buddhi*. *Ashtanga Ghrita* has been prepared as per the Standard Operating Procedures (SOP), mentioned in *Sharangadhara Samhita* for the preparation of *Aushadhi Siddha Ghritas* (Medicated *Ghrita*). The studies on the identity, purity and quality of *Ashtanga Ghrita* will set forth the standard parameter for the preparation of the formulation. Here, efforts have been made to produce safe potent medicated *Ghrita* for *Medhya* effect and establishing the standard parameters for its identity, purity and quality.

Key Words: *Ashtanga Ghrita, Medhya, TLC, Iodine value, Acid value, Saponification value*

Received 05th August 19 Accepted 10th August 21 Published 10th September 2021

INTRODUCTION

Sneha Kalpanas are among the most important secondary dosage forms in *Ayurvedic* pharmaceutics. Crude oils are associated with rancidity factors (*Amadosha*), which are removed and simultaneously therapeutic quality is imparted by the ancient *Ayurvedic* pharmaceutical techniques called *Murchhana*. This *Murchhita Ghrita* was used for preparation of *Ashtanga Ghrita*. *Ashtanga Ghrita* which is mentioned in *Ashtanga Hridaya* for its use in *Medha* (Intellect), *Budhi* (Wisdom), *Vaka* (Speech) and *Smriti* (Memory enhancement). The individual ingredients which are used in the preparation of *Ashtanga Ghrita viz., Brahmi*, *Mandukparni*, *Shatavari*, *Guduchi*, *Vacha*, *Bakuchi*, *Vidhara* and *Shankhpushpi* possess therapeutically proven memory enhancing properties.

AIMS AND OBJECTIVES

a. To prepare *Ashtanga Ghrita* as per SOPs laid down in the classics.

b. To conduct physico-chemical analysis of *Ashtanga Ghrita*.

c. To establish standard parameters for *Ashtanga Ghrita*.

MATERIALS AND METHODS:

ORIGINAL RESEARCH ARTICLE

Murchita Ghrita and ingredients of *Ashtanga Ghrita* were used for the preparation. The samples were tested for physico-chemical analysis like refractive index, specific gravity, saponification value, acid value and iodine value.

Materials:

The following materials were taken for preparation of *Ashtanga Ghrita*.

Method of preparation of *Ashtanga Ghrita*

Apparatus Required:

Weighing balance, wide mouthed vessel, *Khalva Yantra*, Spatula, Cotton cloth, Gas Stove.

Ingredients & their proportions[1]:

Sr. No.	Contents	Part used	Ratio
1.	*Vacha*	Rhizome	1
2.	*Bakuchi*	Fruit	1
3.	*Mandukparni*	Whole Plant	1
4.	*Shankhpushpi*	Whole Plant	1
5.	*Shatavari*	Root	1
6.	*Vidhara*	Stem	1
7.	*Guduchi*	Stem	1
8.	*Brahmi*	Whole Plant	1
9.	*Go-Ghrita*	-	4
10.	*Go-Dugdha*	-	16

Method of Preparation:

Individual plant ingredients of *Kwatha Dravya* were powdered separately in the *Ayurvedic* Pharmacy, Department of *Rasa Shastra* and *Bhaishjya Kalpana*, Faculty of *Ayurveda*, IMS, BHU, Varanasi. *Kwatha* was prepared by the *Kwatha Dravyas* viz., *Vacha, Bakuchi, Vidhara, Guduchi, Shatavari, Mandukparni, Brahmi* and *Vidhara* in the Post Graduate Laboratory of *Rasa Shastra* and *Bhaishjya Kalpana* Department, IMS, BHU, Varanasi. *Murchhita Ghrita* was taken in wide mouthed container which was slightly heated over *Mandagni* till the evaporation of water content, disappearance of froth and sound coming from *Ghrita*, *Kwatha* was added and heating was carried for upto three days. *Go-Dugdha* was added on day 2 of the *Sneha Paka* and heating was done on *Mandagni*. During *Ghrita Paka Madhyamgani* was given for 18 hours 35 minutes (6 hours on 1st day, 7hours on 2ndday and 5hours 35 minutes on 3rd day). *Ghrita Paka* was done till the completion of *Sneha Siddhi Lakshana* was achieved. After obtaining *Sneha Siddhi Lakshana*, the container was taken out of heat and *Ghrita* was filtered through clean cloth in a clean container. The obtained *Ashtanga Ghrita* was stored in a clean, sterile and dry vessel for further use.

Precautions:

a. *Mandagni* was maintained throughout the procedure.

b. *Kalka* was added little by little only after heating the *Ghrita* to check for any spillage.

c. Continuous stirring was carried to avoid sticking of *Kalka* at bottom of pan and carbonization.

d. *Sneha Siddhi Lakshanas* were observed repeatedly and it was confirmed by testing the *Varti* made out of *Kalka*.

e. Care was taken to filter the *Kalka* in warm state itself in order to reduce the loss and to avoid any contamination.

Observations:

Colour of *Ghrita* was changed into greenish brown.

The following materials were taken for the analytical study.

1. *Ashtanga Ghrita* prepared with *Murchita Ghrita*.

2. Electric hot plate with magnetic stirrer.

ORIGINAL RESEARCH ARTICLE

3. Dropper
4. Weighing balance
5. 0.5N Alcoholic KOH solution and 0.5N HCl solution
6. Reflux condenser
7. Water Bath
8. Tituration indicator (phenolphthalein)
9. 0.1N NaOH solution
10. Solvent Ether.
11. Measuring cylinders
12. Pipette
13. Burrate
14. Beaker
15. Round bottom flask
16. Funnel

Methods:

Acid Value

Acid value of an oil or fat is defined as number of milligram of potassium hydroxide required to neutralize the free acid in one gram of sample. This determination should be carried out on fat / oils extracted from sample by continuous extraction with ether.

Method

1. 50 ml of diethyl ether was mixed with 50 ml ethyl alcohol (95%) and one ml of one percentage phenolphthalein solution was added as indicator in the beaker.
2. Then, the obtained solution was neutralized with N/10 potassium hydroxide solution (few drops).
3. About five grams of *Ghrita* was dissolved accurately weighed in mixed neutral solvent and titrated with N/10 potassium hydroxide solution, by continuously shaking, constantly until appearance of pink colour (which persists for fifteen second only) was obtained.
4. The numbers of millilitre required were noted.

The acid value was calculated from the following formula:

$$\text{Acid Value} = \frac{a \times 0.00561 \times 1000}{W}$$

Where 'a' is the number of ml. of 0.1 N potassium hydroxide required, and,

'W' is the weight in g of the substance taken.

Determination of Saponification Value:

1. 35 to 40 g of potassium hydroxide was dissolved in 20 ml water, and sufficient amount of alcohol was added to make 1,000 ml. The mixture was allowed to stand overnight, and the clear liquor was poured off.
2. 2 g of substance was weighed accurately in a tared 250 ml flask; and 25 ml of the alcoholic solution of potassium hydroxide was added to the mixture.
3. Reflux condenser was attached and boiled on a water-bath for one hour, frequently rotating the contents of the flask to cool.
4. 1 ml of solution of phenolphthalein was added to the mixture and the excess of alkali was titrated with 0.5 N hydrochloric acid. The number of ml required (a) were noted.
5. The experiment was repeated with the same quantities of the same reagents in the manner omitting the substance. The number of ml required (b) were noted.

ORIGINAL RESEARCH ARTICLE

The saponification value was calculated from the following formula:—

$$\text{Saponification Value, SV} = \frac{[(b-a) \times 0.02805 \times 1000]}{W}$$

Where 'W' is the weight in g of the substance taken.

Determination of Iodine Value:

The Iodine value of oil is the weight of iodine absorbed by 100 parts by weight of the same, when determined by one of the following method.

1. Oil /fat were accurately weighed in dry iodine flask of capacity 250ml, 10 ml of carbon tetrachloride was added to it and dissolved (the approximate weight in gram of sample to be taken may be calculated by dividing 20 by highest expected iodine value).
2. 10 ml of chloroform and 20 ml of iodine monochloride solution was inserted in the stopper, previously moistened the potassium iodine solution and allowed to stand in a dark place at temperature about 170 ^{o}C for thirty minutes.
3. 15 ml of potassium iodine solution and 100 ml of water was added, shaked and titrated with N/10 sodium thiosulphate using starch mucilage as indicator. The no. of ml required (a) were noted.
4. At the same time the operation in exactly the same manner was carried out but without the sample being tested and the number of ml N/10 sodium thiosulphate required (b) were noted.

$$\text{Iodine Value} = \frac{[(b-a)x\ 0.0269\ x\ 100]}{Weight of\ sample\ in\ grams}$$

Free Fatty Acids

The free fatty acid is the number of ml of sodium hydroxide required to neutralize the fatty acids, resulting from the complete hydrolysis of 5 g of the oil or fat.

Method

1. A solution of sodium hydroxide to be used as an indicator is prepared.
2. The prepared solution was neutralized with 0.5 N sodium hydroxide solutions (few drops).
3. About five grams of *Ghrita* was dissolved accurately weighed in mixed neutral solvent and titrated with N/10 potassium hydroxide solution, by continuous shaking, constantly until a pink colour which persists for fifteen second was obtained.
4. The number of ml required was noted.

Free fatty acids were calculated from the following formula:

$$\text{Free Fatty Acid} = \frac{\text{ml of NaOH used x Normality of NaOH x 2.82}}{W}$$

Where 'W' is the weight in g of the substance taken.

Thin layer chromatography (TLC):

Material required

Coating material (silica gel G), glass plates, oven, solvent system (methanol, chloroform, ethyl acetate, acetic acid etc.), TLC chamber, visualizing reagents (Ethanolic vanillin, Sodium Meta periodate solution, Benzedene solution, Ninhydrine solution).

Preparation of visualizing reagents:

1. Preparation of Sodium Meta periodate ($NaIO_4$) and Benzedene solutions:

ORIGINAL RESEARCH ARTICLE

Solution a: 250 mg of Benzedene was dissolved in 40 ml of ethanol and 10 ml of glacial acetic acid, were mixed properly.

Solution b: 0.5 gm of Sodium Meta periodate was dissolved in 100 ml glacial acetic acid (2 M). Solution a, was applied after five minutes of solution b and obtained the result.

2. Preparation of Anisaldehyde-sulfuric acid (AS) reagent

0.5 ml of anisaldehyde was mixed with 50 ml of glacial acetic acid 1 ml concentrated sulphuric acid was added to it. After sprayed the plate was heated at 100^0C for 6 minutes.

Method

At first silica gel and water were mixed together to make slurry. Then, the slurry was uniformly spread on the plates and plate was air dried for 30 minutes then kept the plate in hot air oven at 110-120^0C for 30 minutes. The plate was taken out from the hot air oven. Next with the help of capillary tubes the samples were applied on the one end of the plate. The plate was air dried then poured in the pre saturated TLC chamber. The development was stopped when solvent front reached to $3/4^{th}$ of the plate. The plate was taken out from chamber and air dried and solvent front marked. Next the plate was dried and spray visualizing agent. The R_f value measured from the plate by using formula:

R_f=(Distance travelled by solute from origin) / (Distance travelled by solvent from origin)

Test for Heavy Metals

Limit test for heavy metals was done by Atomic Absorption Spectrophotometer (AAS).

Atomic Absorption Spectrophotometry

Atomic absorption spectrophotometer is used in the determination of heavy metal elements and some non-metal elements in the atomic state.

The light of characteristic wave length emitted from a cathodic discharge lamp is absorbed when it passes through the atomic vapour generated from sample containing the element being examined atomized to the ground state. The assay of the element being examined is tested to determine the decreased degree of light intensity of radiation.

Atomic absorption obeys the general rule for absorption spectrophotometry. The assay is carried out by comparing the absorbance of the test preparation with that of the reference preparation.

Determination of Lead, Pb (Graphite Oven Method)

i.) Determination conditions

The following ideal conditions were maintained:

- Dry temperature: 100-120ºC, 20 seconds
- Ash temperature: 400-750ºC, 20-25 seconds
- Atomic temperature: 1700-2100ºC, 4-5 seconds;
- Measurement of wavelength: 283.3 nm;
- Background calibration: deuterium lamp (D lamp) or Zeeman effect.

ii.) Preparation of lead standard stock solution

A quantity of lead single-element standard solution was measured accurately to prepare standard stock solution with 2% nitric acid

solution, which containing 1 µg per ml, and was stored at 0-5ºC.

iii.) Preparation of calibration curve

A quantity of lead standard stock solutions was measured respectively, diluted with 2% nitric acid solution to the concentration of 0, 5, 20, 40, 60, 80 ng per ml, respectively. 1 ml of the above solution was measured accurately and 1ml of 1% ammonium dihydrogen phosphate (ADP, $NH_4H_2PO_4$) and 0.2% magnesium nitrate [$Mg(NO_3)_2$] were added and mixed well, pipette accurately 20 µl to inject into the atomic generator of graphite oven and their absorbance was determined, then, the calibration curve was drawn with absorbance as vertical axis and concentration as horizontal ordinate.

iv.) Preparation of test solution

- About 0.5 g of the coarse powder of the substance was weighed accurately for examination and transferred into a casparian flask.
- 5-10 ml of the mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) (4 : 1) was added, a small hopper on the flask-top was added, and the mixture was macerated overnight.
- Heated to slake on the electric hot plate, somewhat-boiling temperature was maintained, when brownish-black in colour appeared, then, again a quantity of the above mixture was added, continuously heat was given till the solution became clear and transparent, then the temperature was raised, heat was continuously given to thick smoke, till white smoke dispersed, the slaked solution became colourless and transparent or a little yellow, when cool, it was transferred into a 50 ml volumetric flask.
- The container with 2% nitric acid solution (HNO_3) was washed, washing solution was added into the same volumetric flask and diluted with the same solvent to the volume, the mixture was shaked well.
- Synchronously the reagent blank solution was prepared according to the above mentioned procedure.

v.) Determination

- 1 ml of the test solution was measured accurately and its corresponding reagent blank solution respectively.
- 1 ml of solution containing 1% $NH_4H_2PO_4$ and 0.2% Mg $(NO_3)_2$ was added and shaked well, pipette accurately 10-20 µl to determine their absorbance according to the above method of preparation of calibration curve.
- The content of lead (Pd) in the test solution was calculated from the calibration curve.

Determination of Cadmium (Cd) (Graphite Oven Method)

i.) Determination conditions

Following ideal conditions were followed.

- Dry temperature: 100-120ºC, 20 seconds.
- Ash temperature: 300-500ºC, 20-25 seconds.
- Atomic temperature: 1500-1900ºC, 4-5 seconds
- Measurement wavelength: 228.8 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

ORIGINAL RESEARCH ARTICLE

ii.) Preparation of Cd standard stock solution

A quantity of Cd single-element standard solution was measured accurately to prepare standard stock solution Cd with 2% HNO_3, which was containing 0.4 µg per ml Cd, stored at 0-5ºC.

iii.) Preparation of calibration curve

- A quantity of cadmium standard stock solutions was measured accurately, diluted to the concentration of 1.6, 3.2, 4.8, 6.4 and 8.0 ng per ml with 2% HNO_3 respectively.
- 10 µl of the above solutions were pipette accurately respectively and further injected them into the graphite oven.
- Their absorbance was determined and then the calibration curve was drawn with absorbance as vertical axis and concentration as horizontal ordinate.

iv.) Preparation of test solution

The same method of preparation of test solution was used for Pb as above.

v.) Determination

- 10-20 µl of the test solution was pipetted accurately and its corresponding reagent blank solution respectively.
- Their absorbance was determined according to the above method of preparation of calibration curve.
- When interference occurs then,1 ml of the standard solution, blank solution and test solution were weighed accurately respectively.
- 1 ml of a solution containing 1% $NH_4H_2PO_4$ and 0.2% Mg $(NO_3)_2$ were added and shaked well.
- Their absorbance was determined according to the method mentioned above.
- The content of Cd in the test solution was calculated from the calibration curve.

Table 1 Results of Phytochemicals

Phyto chemical	Stationary phase	Mobile phase	Visualizing Reagents	Results
Terpenoid	Silica gel	Chloroform: Methanol (9:1)	Anisaldehyde	Blue colour
Glycoside	Silica gel	Butanol: acetic acid: water (4: 2: 1)	Sodium-meta periodite and benzedene	White spot in blue back ground
Saponin	Silica gel	Chloroform: Methanol (7: 3)	Ethanolic vanillin and ethanolic sulfuric acid	Blue colour

RESULTS

- **Thin Layer Chromatography**
- Solvent System: Toluene: Ethyl Acetate: Acetic acid: 8:2:1
- Spraying Agent: Anisaldehyde Sulphuric acid. The results of thin layer chromatography are mentioned in table no 1, 2 and 3 respectively.

Table 2 Preliminary Phytochemical Investigation of Ethanolic Extract

Serial No	Phytoconstituents	Ethanolic extract *AshtangaGhrita*
1.	**Alkaloids**	-
2.	**Phenolic compound**	+
3.	**Terpenes (sesquiterpens)**	-
4.	**Terpenes (di & tri terpens)**	+
5.	**Glycoside**	+
6.	**Flavonoids**	-
7.	**Steroids**	+
8.	**Amino acids**	+
9.	**Volatile oil**	+

ORIGINAL RESEARCH ARTICLE

Table 3 The R_f values of phytochemicals in ethanolic extract

Phytochemicals	Plant extracts	Mobile Phase	R_f value
Flavonoids	EtOH	$CHCl_3$:EtOH (9:1)	Absent
Alkaloids	EtOH	$CHCl_3$:EtOH (9:1)	Absent
Phenolic compound	EtOH	$CHCl_3$:EtOH (9:1)	0.61, 0.57, 0.64, 0.66
Amino acids	EtOH	$CHCl_3$:EtOH :NH_3 (4:4:2)	0.33, 036, 0.45, 0.36
Glycoside	EtOH	Butanol: Acetic acid: water (4:2:1)	0.42
Steroids	EtOH	Pet ether : EtOAc (8:2)	0.38, 0.35

Note: No Spots were seen under visible light before and after spray in this sample.

The values of analytical parameters viz., acid value, saponification value, iodine value, refractive index and free fatty acids of *Ashtanga Ghrita* are mentioned in table no. 4

Table 4 Values of Analytical Parameters for *Ashtanga Ghrita*

Sample	Acid Value	Saponification Value	Iodine Values	Refractive Index	Free Fatty Acid
Ashtanga Ghrita	5.04	238.4	35.40	1.453	0.25

The characterstic features in the organoleptic values of *Ashtanga Ghrita* are mentioned in table no. 5

Table 5: Organoleptic Values of *Ashtanga Ghrita.*

Sample	Colour	Taste	Odour	Touch	Appearance
Ashtanga Ghrita	Yellowish Green	Acrid	Characteristic	Smooth and Greasy	Viscous and Semisolid

The values of heavy metal analysis for lead and cadmium of *Ashtanga Ghrita* are mentioned in table no. 6

Table 6 Values of Heavy Metals for *Ashtanga Ghrita*

Sample	Lead	Cadmium
Ashtanga Ghrita	Less than 0.4696 ppm	Less than 0.0029 ppm

OBSERVATION AND DISCUSSION

There is an increase in the use of Traditional Medicine (TM) and Complementary and Alternative Medicine (CAM) in developed as well as developing countries due to its lesser or no side effects. The phyto-medicinal therapy is easy to procure and administer in different pharmaceutical dosage forms like *Churna*, *Vati*, *Taila*, *Ghrita* etc. The ingredients of *Ashtanga Ghrita* have been proved scientifically effective for their role in enhancing the intellect and memory enhancing properties.

In present work, an extensive chemical and chromatographically analysis of *Ashtanga Ghrita*

ORIGINAL RESEARCH ARTICLE

was done. The main objective of the study was to establish the standard parameters for this noble medicine. The characteristic physical and chemical parameters were carried out, such as, Iodine Value, Saponification value, Acid value, Free Fatty Acids, Heavy Metals and Thin Layer Chromatography.

In the above analytical findings, it is observed that synergism of the specific components and application of ayurvedic pharmaceutical procedures is done in formulations and thus it reflects to produce different pharmacological actions. It is observed that *Ashtanga Ghrita* was non-sticky in touch, yellowish green in colour, the odour was acrid natured and the taste was astringent-bitter.

Acid value is called as neutralization number. This is the amount of potassium hydroxide required in milligram to neutralize the acid present with any compound. Thus, this value indicates the Free Fatty Acids (FFAs) present in *Ashtanga Ghrita*. It is related to its stability and shelf life. Formation of FFAs might be the important factor for rancidity of *Ghrita*. FFAs is formed due to the hydrolysis of triglycerides which get promoted by the reaction with moisture present in Ghrita.[2,3]

Iodine value determines the amount of unsaturated fatty acids in double bond form which reacts with iodine. Higher iodine values indicate that fats are rich in poly-unsaturated fatty acids (PUFA) which is helpful in reducing LDL cholesterol level[4]. These are lipid base formulations, due to presence of PUFA, those formulations do not alter or change physiological lipid profiles of human being.

Saponification value is the measure of average molecular weight (or chain length) of all fatty acids present in the fats[5]. It is said that in manufacture of medicated *Ghrita* formulations, because of the process of hydrolysis, release of lower molecular weight SCFA (short chain fatty acids) is carried out. It might be happening in the presence of alkaline nature of paste ofherbal drugs and liquid media. SCFA are recognised as an essential fuel source for colonocytes, particularly in distal colon. The histological, endoscopic and metabolic similarity between diversion colitis and ulcerative colitis suggests that nutritional SCFA deficiency state play an important role in pathogenesis of these diseases[6]. SCFA are readily absorbed and may play an important role as a protective effect for distal colon[7]. It can be inferred that *Ashtanga Ghrita* may improve colonic health as it might be getting easily absorbed as well as digested. It can possibly play a protective role.

Peroxide value: Peroxides are intermediate products during fat oxidation. Rapid breakdown takes place to aldehydes, ketones and other products. This value is indicative of fat oxidation. It is used to check the stability and rancidity of fats by measuring lipid peroxides and hydro-peroxides formed during the initial stages of oxidation[3,4].

CONCLUSION

In the present study, analytical values for *Ashtanga Ghrita* are established. Analytical data

generated for *Ashtanga Ghrita* prepared from *Murchitta Ghrita* will possibly be considered as quality standard. From above analytical data it can be inferred that *AshtangaGhrita* is safer for internal administration for *Medhya* disorders. Further, researchers can use these analytical values of *Ashtanga Ghrita* as standard values. A detail further research is needed for scientific validation and further establishment of data by using higher instrumental techniques.

REFERENCES

1. Vagbhatacharya, *Ashtanga Hridaya*, Uttar Sthana 1, Balopcharniya 43-44, Commentator Atrideva Gupta, Author Yadunandana Upadhyaya, Publisher Chaukhambha Prakashana, 619p.

2. Frega N, Mozzon M, Lercker G.1999. Effects of free fatty acids on oxidative stability of vegetable oil. Journal of American Oil Chemists' Society, 76(3):325-329

3. Haldar Pronab, Mahapatra BN, Agarwal DS, Singh AK. 2013. Pharmaceutico analytical study and standardisation of Panchatikta ghrita. International Research Journal of Pharmacy, 4(9): 173-179.

4. Sharma VK, Verma D, Sharma U, Rohila R, Pandey NN. 2016. Applicability of physicochemical parameters in stability and shelf-life estimation of ayurvedic semisolid dosage form 'Ashtamangalghrita' at accelerated storage conditions, Research Journal of Pharmaceutical, Biological and Chemical sciences, 7(1):170-182.

5. Kadibagil VR, Sarashetti RS. 2017. Assessment of significant role of murcchan samskara of ghrita by physico-chemical analysis. International Journal of Research in Ayurveda and Pharmacy, 8(Suppl 2):162- 165.

6. Rabassa AA, Rogers AI. 1992. The role of short chain fatty acid metabolism in colonic disorders, American Journal of Gastroenterology, 87(4):419-423

7. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. 2006. Colonic health: Fermentation and short chain fatty acids. Journal of clinical Gastroenterology, 40(3):235-243.