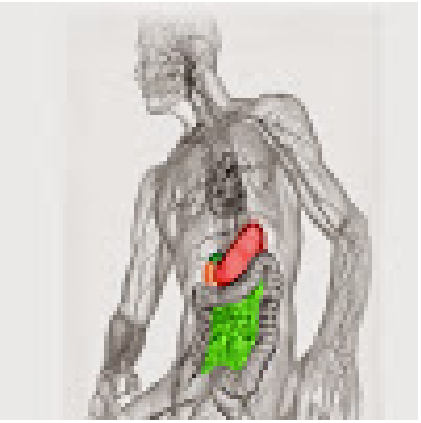

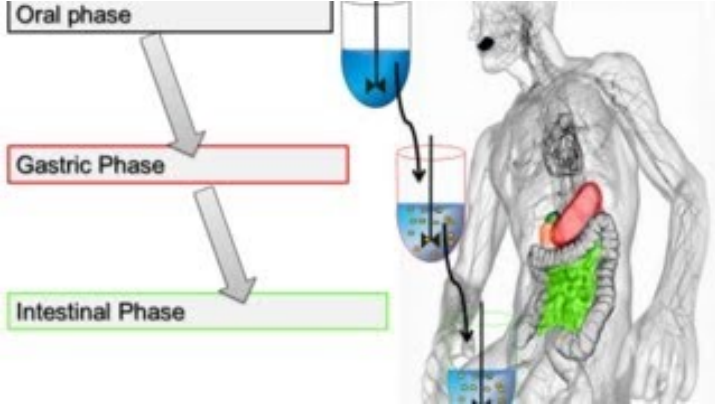

The Binding of Bile Acids by Biscuits with Bioactive Substances during *In Vitro* Digestion

Krzysztof Dziedzic, Danuta Górecka, Artur Szwengiel, Marzanna Hęś, Patrycja Komolka

Poznan University of Life Sciences

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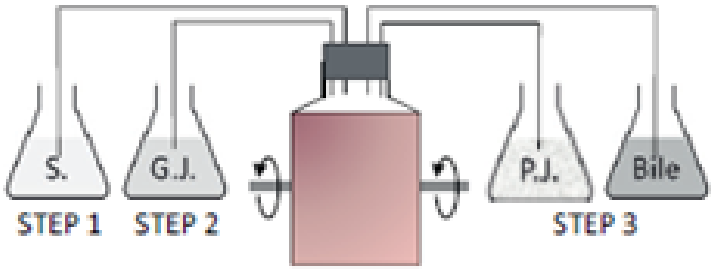
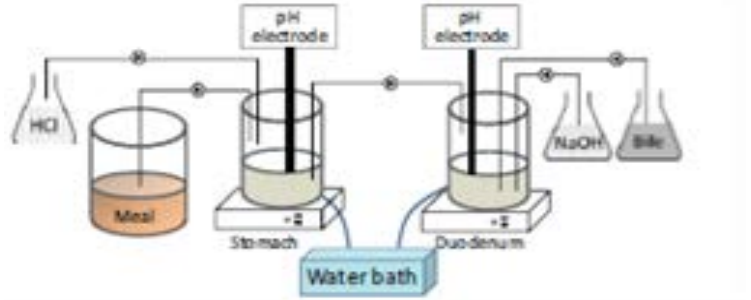
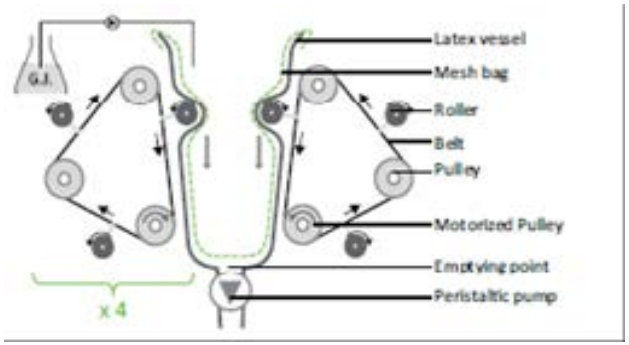
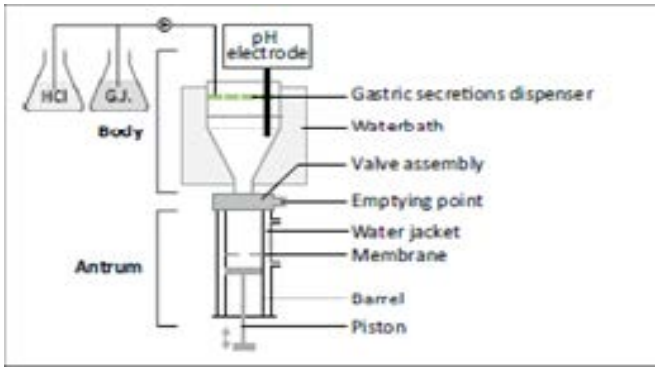
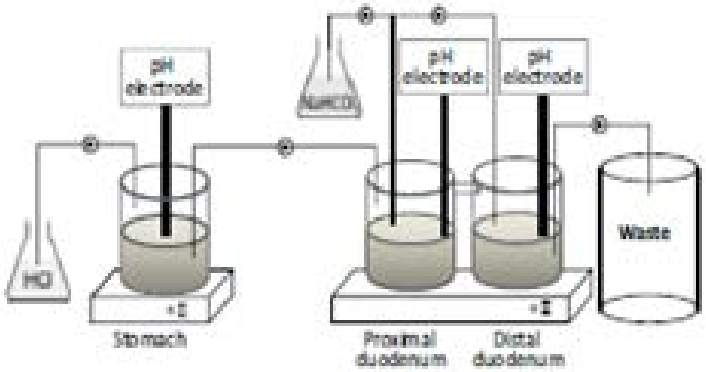
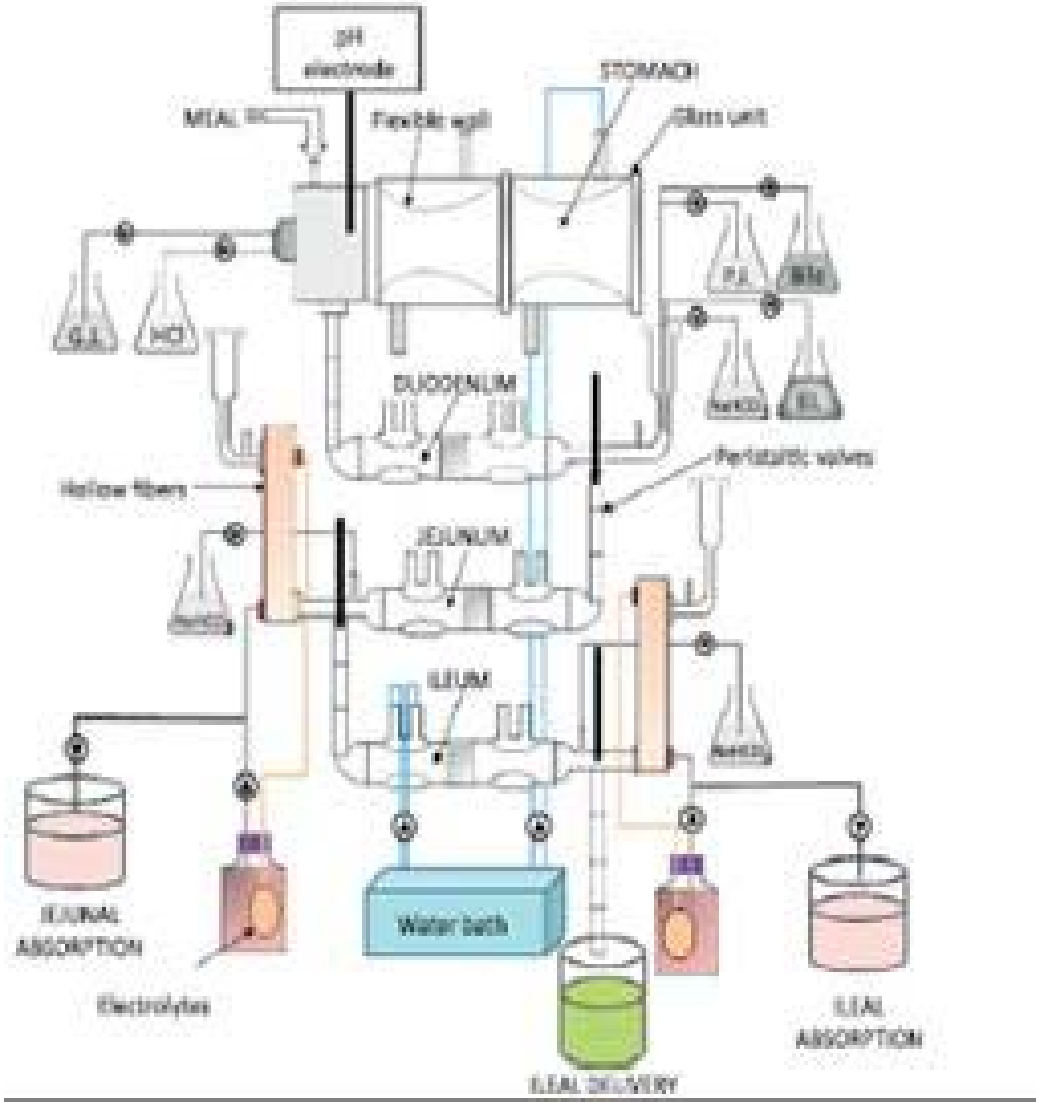

S.
G.J.
P.J.
Bile
STEP 1
STEP 2
STEP 3


pH electrode
pH electrode
HCl
NaOH
Bile
Meal
Stomach
Duodenum
Water bath

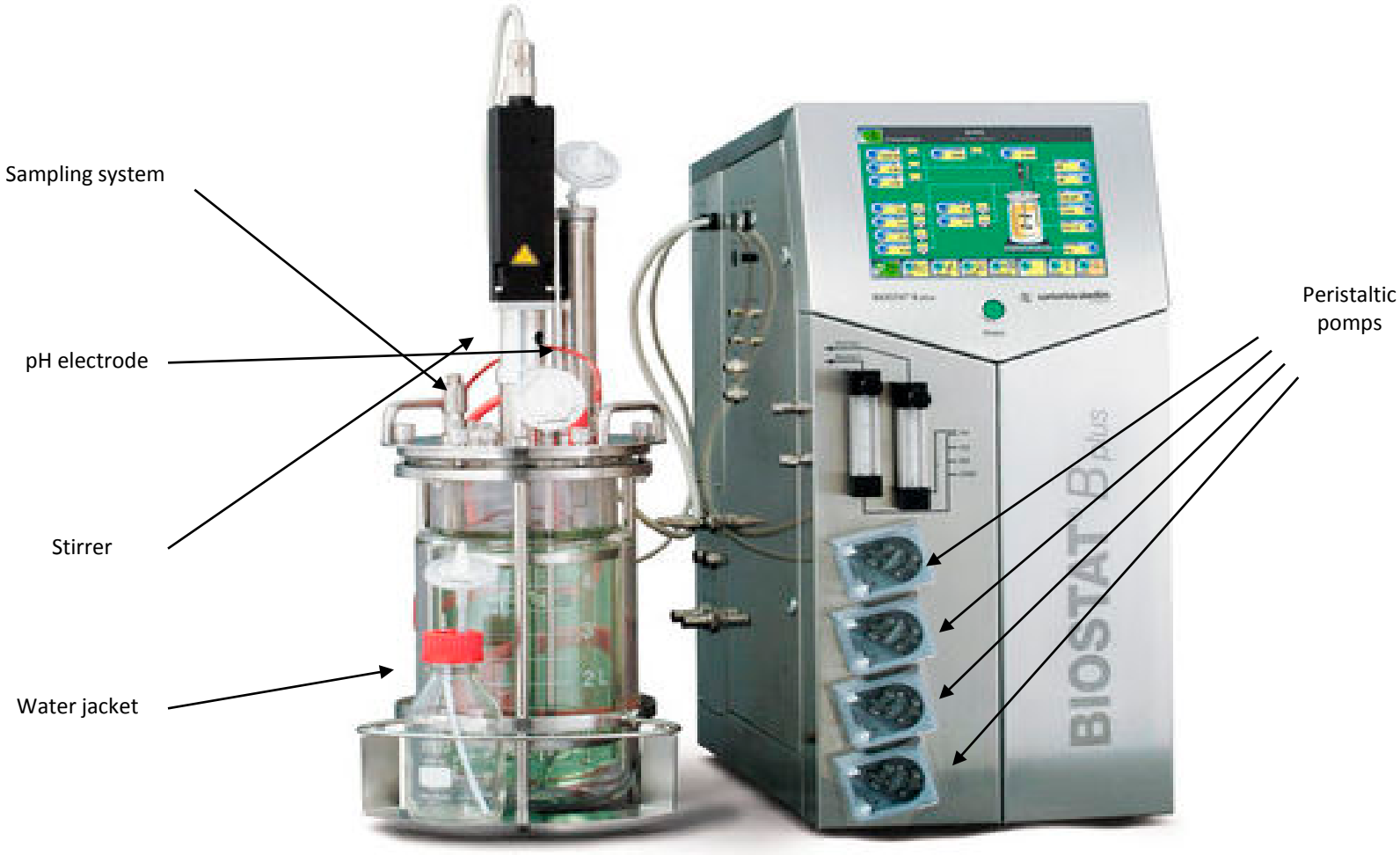

G.J.
Latex vessel
Mesh bag
Roller
Belt
Pulley
Motorized Pulley
Emptying point
Peristaltic pump
x 4

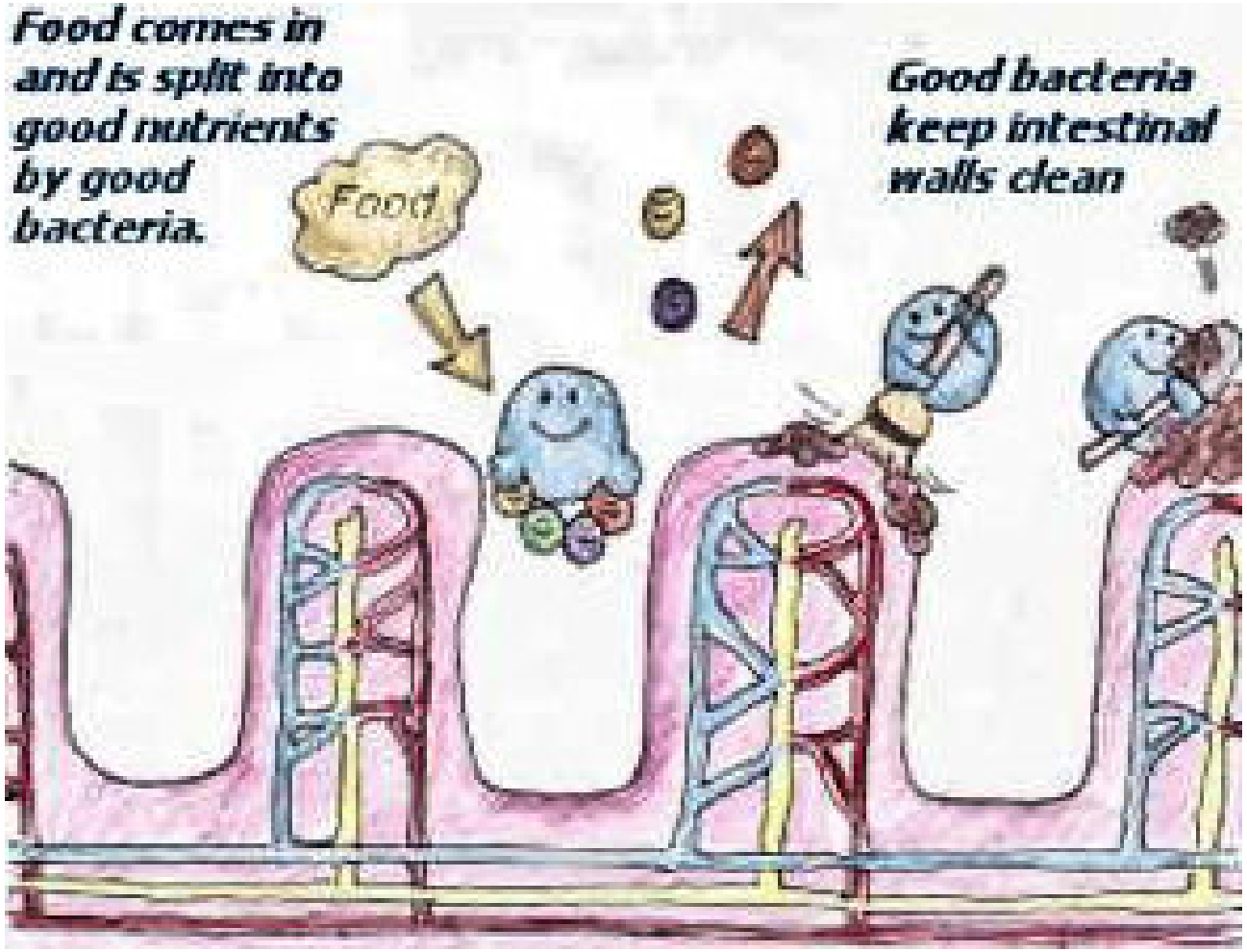

pH electrode
HCl
G.J.
Body
Antrum
Gastric secretions dispenser
Waterbath
Valve assembly
Emptying point
Water jacket
Membrane
Barrel
Piston


pH electrode
pH electrode
pH electrode
HCl
Waste
Stomach
Proximal duodenum
Distal duodenum

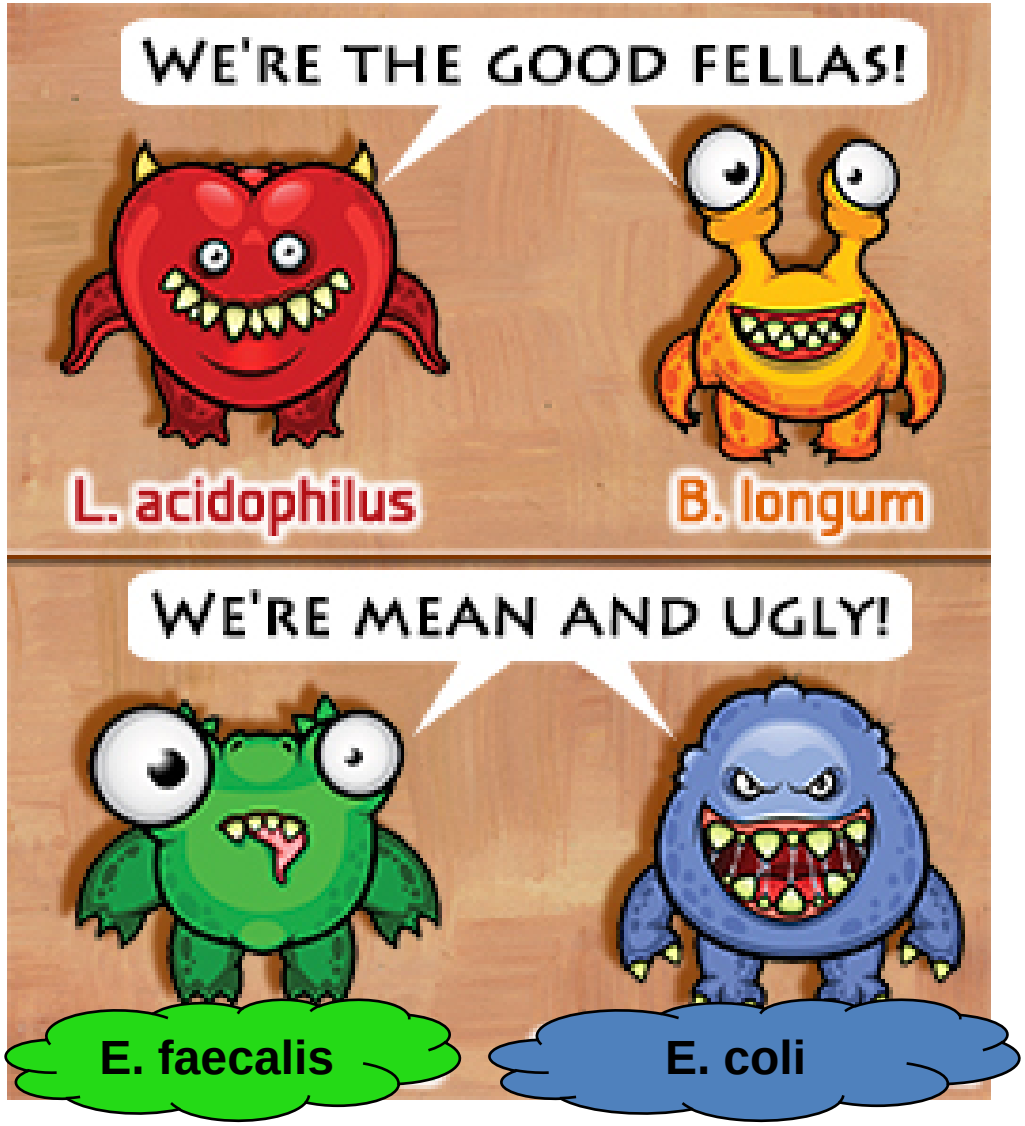

pH electrode
STOMACH
MEAL
Flexible wall
Glass unit
G.A.
HCl
P.E.
BM
DUODENUM
Peristaltic valves
Hollow fibers
JEJUNUM
ILEUM
JEJUNAL ABSORPTION
Electrolytes
Water bath
ILEAL ABSORPTION
ILEAL DELIVERY

Bioreactor used during in vitro digestion-singel chamber static model

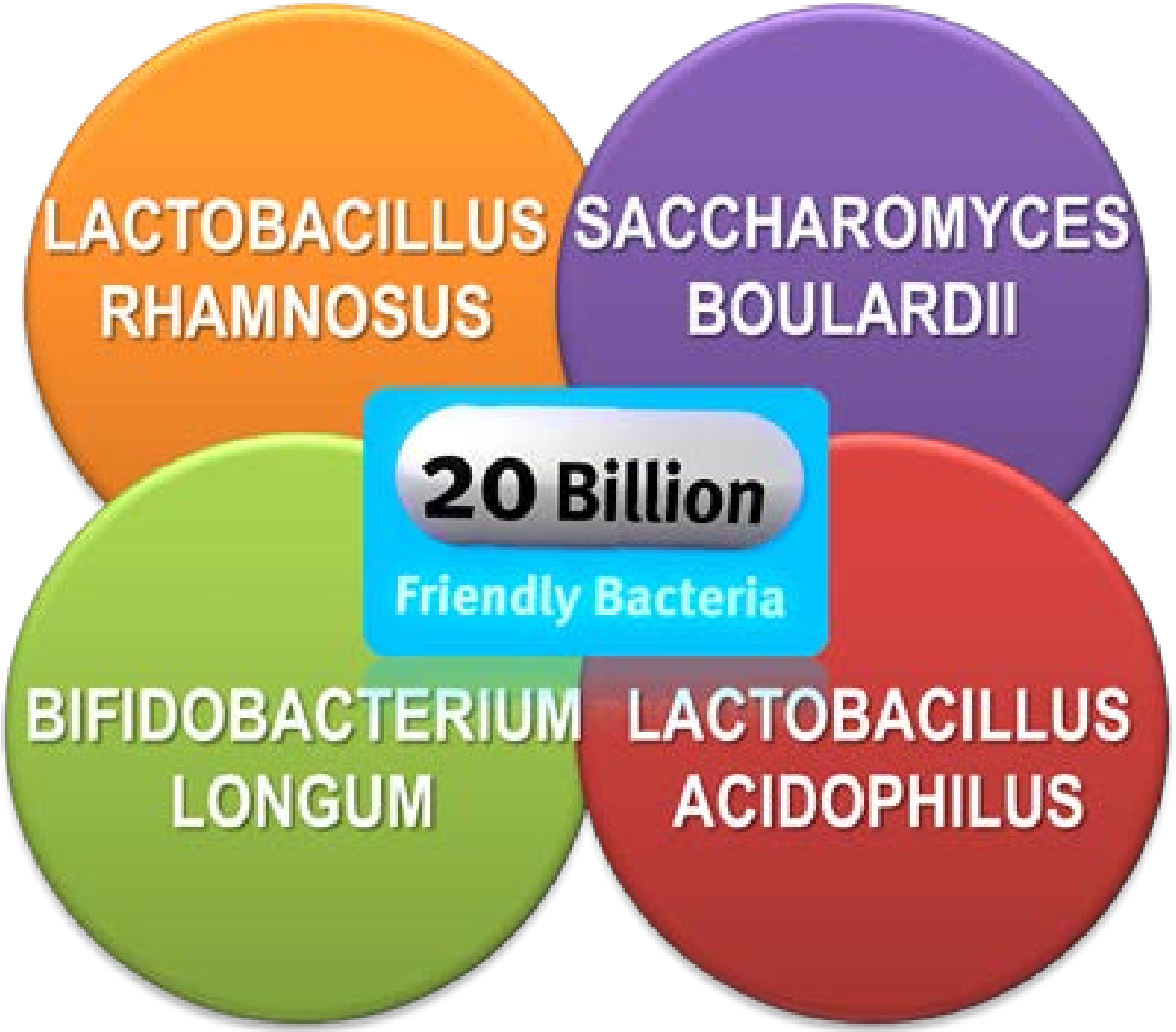




www.naturalvigour.com



www.buzzle.com



Typical Intestinal Bacteria

Classification	Representative bacteria	Action	Effects on body
Beneficial (good) bacteria	• *Bifidobacteria* • Lactic acid bacteria	Vitamin synthesis Digestion and absorption assistance Infection prevention Immunity stimulation	Health maintenance Anti-aging
Harmful (bad) bacteria	• *Clostridium perfringens* • *Staphylococcus* • *E.coli (toxic strain)*	Intestinal putrefaction Production of bacterial toxin Production of carcinogenic substances Gas production	Health inhibition Trigger of disease Promotion of aging
Opportunistic bacteria	• *Bacteroidetes* • *E.coli (nontoxic strain)* • *Streptococcus*		No trouble when healthy, but have adverse actions inside the intestines when the body is weak

www.otsuka.co.jp

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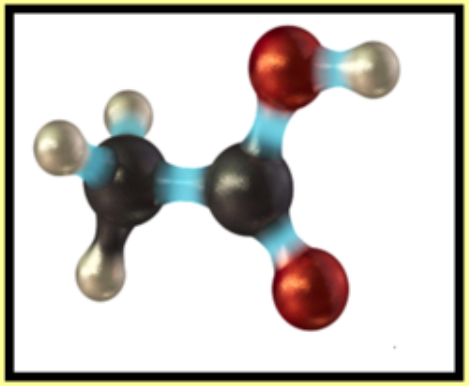
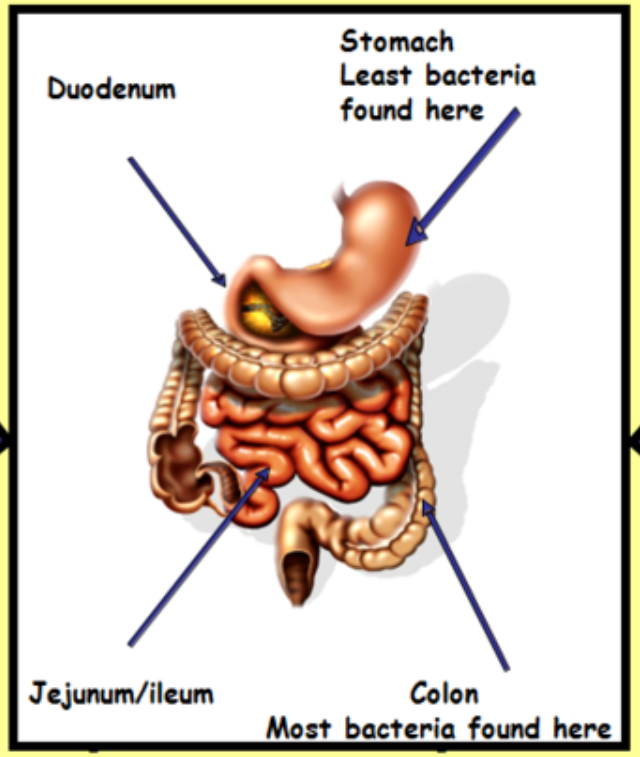
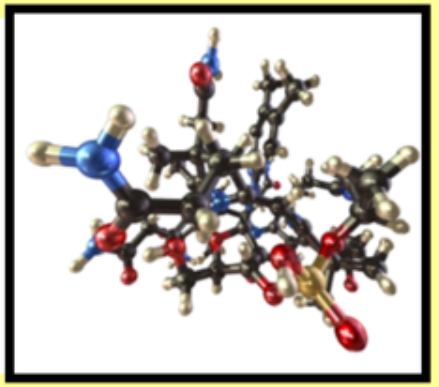


Digestion of food and the production of beneficial metabolites e.g. acetic acid



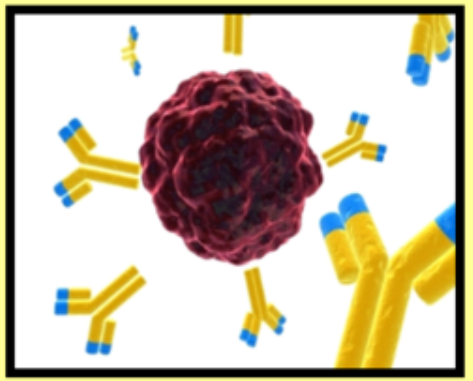


Vitamin synthesis (K and B12 vitamins)

Development of the intestinal structure

Metabolism of toxic compounds

Immune system development



The aim of study

determination of bile salts binding ability by pastry goods during *in vitro* digestion

- **Dietary fiber assays**:
  - Total dietary fiber
  - Insoluble dietary fiber
  - Soluble dietary fiber
  - Neutral detergent fiber
  - Cellulose
  - Hemicellulose
  - Lignin
- **Bile salts assays:**
  - Cholic acid
  - Deoksycholic acid
  - Lithocholic acid

Methods

- Neutral detergent fiber, cellulose (C), hemicellulose (H) and lignin (L) was assayed using Van Soest method;
- Total dietary fiber (TDF), soluble (SDF) and insoluble (IDF) fractions were assayed using Asp method
- The ability to bind bile acids was estimated using UHPLC equipment

1. Van Soest, P.J. 1963. Use of detergents in the analysis fibrous feeds. I. Preparation of fiber residues of low nitrogen content. J. AOAC Int. 46: 825-835.

2. Van Soest, P.J. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. J. AOAC Int. 50: 50-55.

3. Asp N.-G., Johansson C.-G., Hallmer H. and Siljestrom M. 1983. Rapid enzymatic assay of insoluble, and soluble dietary fiber. J. Agr. Food Chem. 31: 476-482.

4. Asp N.-G. 1996. Dietary carbohydrates: classification by chemistry and physiology. Food Chem. 57: 9-14.

5. Wang, W., Onnagawa, M., Yoshie, Y., Szuzuki, T. 2001. Binding of bile salts soluble and insoluble dietary fibers of seaweeds. Fishieries Science. 67: 1169-1173.

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The Samples

- Control biscuits (CB)
- Bioactive biscuits (BB1)
- Bioactive biscuits (BB2)

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Table 1. Ingredients of biscuits.

	CB	BB1	BB2
Plant butter	20	10	10
Innulin	-	2	2
Water	-	4	4
Egs	20	21	21
Sugar	17	17	17
Wheat flour	43	23	33
Buckwheat flour	-	21	11
Buckwheat hull	-	2	2

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Model of research

	Organic acids	Phenolic compounds	Bile salts	carbohydrates
Stomach (pH 2,0; after 10min)	x	x		x
Stomach (after 2h)	x	x		x
Small intestine (pH 6,0; after 30 min)			x	
Small intestine (pH 7,4)	x	x	x	x
Large intestine (pH 8.0; start)	x	x	x	x
Large intestine (pH 8.0; final)	x	x	x	x

Model of research

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Small intestine (pH 7,4)	x	x	x	x
Large intestine (pH 8.0; start)	x	x	x	x
Large intestine (pH 8.0; final)	x	x	x	x

MORFOLOGIA KRWI OBWODOWEJ

LEUKOCYTY	7,80x10^3/uL		4,40 — 11,30x10^3/uL
NEUTROCYTY %	51,4	%	45,0 — 70,0 %
LIMFOCYTY %	36,6	%	19,0 — 48,0
MXD %	12,0	%	↑ 0,0 — 10,0
NEUTROCYTY	4,00x10^3/uL		1,80 — 7,70
LIMFOCYTY	2,90x10^3/uL		1,00 — 4,50
MXD	0,90x10^3/uL		0,00 — 1,00
ERYTROCYTY	5,19x10^6/uL		3,80 — 5,60
HEMOGLOBINA	14,2	g/dl	12,4 — 17,2
HEMATOKRYT	44,0	%	38,0 — 50,0
MCV	84,8	fL	80,0 — 100,0
MCH	27,4	pg	27,0 — 34,0 fL
MCHC	32,3	g/dl	32,0 — 37,0 g/dl
RDW	16,0	%	↑ 11,5 — 14,5 %
PLYTKI	246,0x10^3/uL		130 — 440 x10^3/uL
MPV	10,4	fL	7,2 — 11,1
PDW	11,6		
P-LCR	27,6		

Opis

Flagi
P-LCR:sld

Symptomy	Liczba przypadków	Całkowita eliminacja	Wyraźne działanie	Dostateczne działanie	Niewielki efekt
Bóle stawów	33	30,30 %	45,50 %	15,20 %	9,00 %
Bóle dolnych partii nóg	35	40,00 %	22,86 %	22,86 %	14,28 %
Bóle reumatyczne	32	43,75 %	21,88 %	25,00 %	9,37 %
Bóle ramion	30	16,67 %	33,33 %	20,00 %	30,00 %
Bóle kręgosłupa	33	24,24 %	15,15 %	27,27 %	33,34 %
Bóle pleców	30	16,67 %	33,33 %	30,00 %	20,00 %
Bolesne miesiączkowanie	32	40,62 %	21,88 %	15,62 %	21,88 %
Owrzodzenia i czyraki	35	42,86 %	14,28 %	28,58 %	14,28 %
W sumie	260	32,30 %	25,77 %	23,08 %	18,85 %

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Results

Tab.1. Content of neutral dietary fiber (NDF) and its fraction.

Fraction \ Sample	Control biscuits (CB)	Bioactive biscuits (BB1)	Bioactive biscuits (BB2)
NDF	3.53^{b}	5.20^{a}	4.83^{c}
Cellulose	0.95^{c}	1.99^{ab}	1.37^{bc}
Hemicellulose	0.78^{bc}	0.84^{ab}	0.20^{c}
Lignin	1.80^{c}	2.38^{b}	3.26^{a}

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Tab.2. Content of total dietary fiber (TDF) and its fractions.

Sample / Fraction	Control biscuits (CB)	Bioactive biscuits (BB1)	Bioactive biscuits (BB2)
Total Dietary Fiber	8.7^{c}	11.2^{ab}	12.8^{a}
Insoluble Dietary Fiber	4.5^{c}	7.5^{a}	7.4^{ab}
Soluble Dietary Fiber	4.2^{b}	3.8^{c}	5.4^{a}

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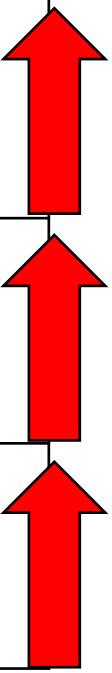

Tab.3. Bile acid binding capacity by pastry goods.

		Control (mg/mL)	**CB** (mg/mL)	**BB1** (mg/mL)	**BB2** (mg/mL)
Cholic acid	Small intestine	3265	2257 (-31%)	3087 (-5%)	274 (-92%)
	Large intestine	3055	3458 (+13%)	3332 (+9%)	1250 (-59%)
Deoksycholic acid	Small intestine	11181	848 (-92%)	765 (-93%)	29 (-99%)
	Large intestine	2696	3527 (+31%)	3136 (+16%)	301 (-89%)
Lithocholic acid	Small intestine	320	91 (-72%)	126 (-61%)	182 (-56%)
	Large intestine	2223	2675 (+20%)	3576 (+61%)	3458 (+56%)

„-” means decrease of bile acid in comparison to control sample , „+” means increase of bile acid in comparison to control sample.

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Tab.3. Bile acid binding capacity by pastry goods.

		Control (mg/mL)	CB (mg/mL)	BB1 (mg/mL)	BB2 (mg/mL)
Cholic acid	Small intestine	3265	2257 (-31%)	3087 (-5%)	274 (-92%)
	Large intestine	3055	**3458 (+13%)**	**3332 (+9%)**	**1250 (-59%)**
Deoksycholic acid	Small intestine	11181	848 (-92%)	765 (-93%)	29 (-99%)
	Large intestine	2696	**3527 (+31%)**	**3136 (+16%)**	**301 (-89%)**
Lithocholic acid	Small intestine	320	91 (-72%)	126 (-61%)	182 (-56%)
	Large intestine	2223	**2675 (+20%)**	**3576 (+61%)**	**3458 (+56%)**

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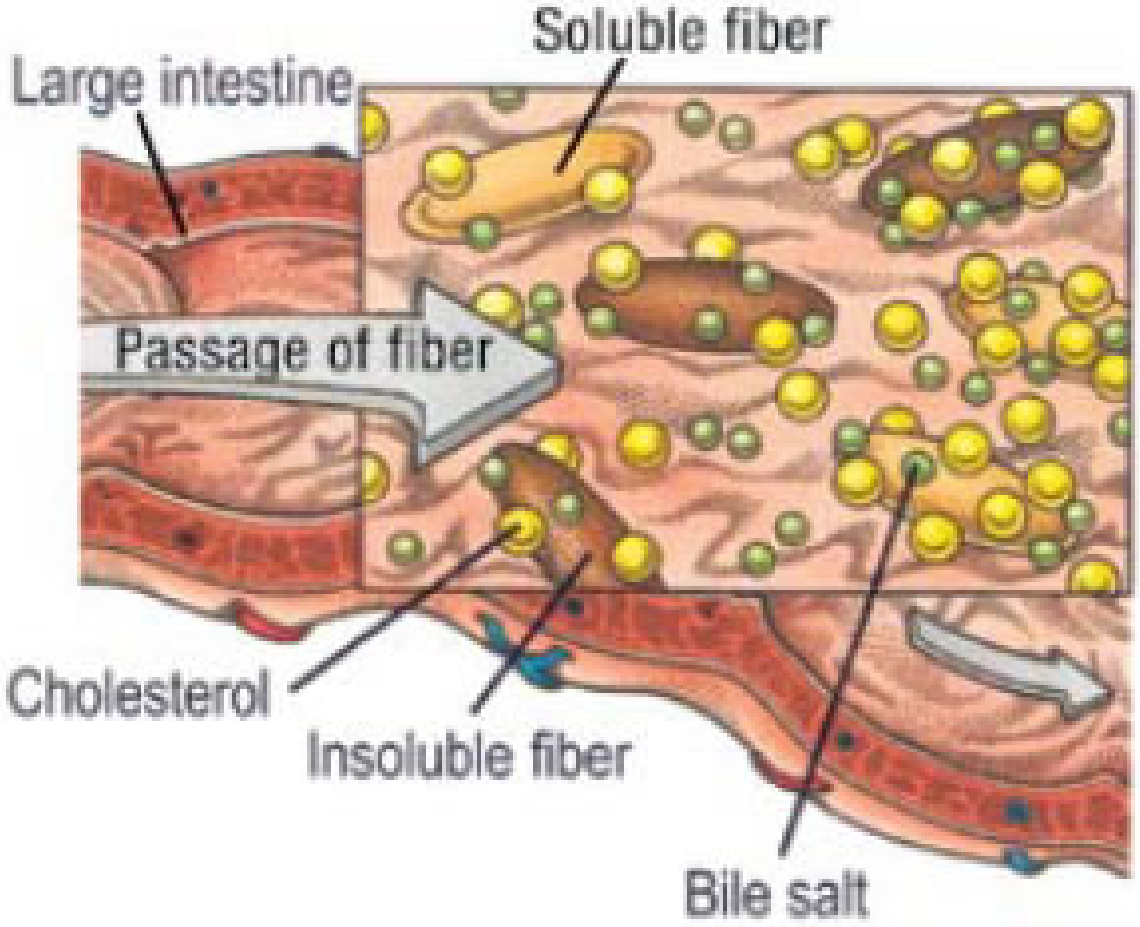

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Conclusions

- Biscuits containing bioactive ingredients were characterized by a higher content of NDF and TDF dietary fibre, as compared with the control samples
- It was found that the ability to bind bile acids depended both on the type of tested product and the type of bile acid
- The highest cholic and deoksycholic acid binding ability was observed in case of bioactive biscuits 2
- The content of cholic, deoksycholic and lithocholic acid in the large intestine section **increased** in case of control biscuits and bioactive biscuits 1.

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Thank You